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GENETIC SEGREGATION[1]

W. BATESON, M.A., F.R.S.

DIRECTOR OF THE JOHN INNES HORTICULTURAL INSTITUTION

THE later developments of Mendelian analysis have been in the main an attempt to elucidate the scope and nature of segregation. Mendel proved the existence of characters determined by integral or unit factors. Their integrity is maintained by segregation, the capacity, namely, to separate unimpaired after combination with their opposites. Our first aim has been to discover specifically what characters behave in this way, whether there is any limit to the scope of segregation, or any characters or classes of characters which are determined by elements unable to segregate simply. The second object has been to decide the time and place in the various life-cycles at which segregation occurs. It is with the latter problem that I propose to deal more particularly in this lecture, but a brief consideration of the range of characters, subject to segregation, is appropriate.

1. THE SCOPE OF SEGREGATION

Of the classes of features by which animals and plants are distinguished, most have now been shown to be dependent on segregable elements. It is perhaps noticeable that we have no quite clear proof that the factors governing differences in number, meristic characters in the strict sense, commonly behave so simply as those determining other characteristics. There are examples of parts repeated in series, such as the extra toe of the

[1] Croonian Lecture delivered before the Royal Society of London, June 17, 1920, and printed in the *Proceedings*.

fowl (a dominant) and the leaf of the monophyllous strawberry with a single leaflet (a recessive) which have a factorial inheritance, but the resulting terms, especially the heterozygotes, are indefinite. In the polydactylous fowl, for example, the heterozygote has a great variety of shapes. The hind toe is most often represented by two sub-equal digits, but the duplicity may be so slight as to appear externally only as a division of the nail. It may also assume another very different form, in which there is only a single many-jointed digit representing the usual pair. In the monophyllous strawberry the homozygous recessive, whether before or after immediate extraction from the heterozygote, shows fluctuations to a 2- or 3-leaved condition.[2] Such fluctuations are common among forms distinguished by meristic peculiarities. There are not the uniformity and simplicity which are such striking features of variations in color and many other substantive characteristics. The evidence as to meristic differences is, however, still scanty, and it is too soon to decide what importance should be attached to this preliminary impression.

With more confidence we recognize that merely quantitative differences seldom, if ever, have a perfectly simple inheritance. There are two obvious interpretations: (1) that the factors do not usually segregate clean; (2) that the number of factors involved is so large that their effects are masked. Adequate discussion of these two possibilities could only be given at considerable length. On the whole, I incline to the former alternative, but the material for a decision scarcely exists as yet. Certain examples should be noticed in which, though the most obvious differentiating feature is quantitative, the underlying physiological distinction is more probably to be referred to a qualitative factor. Height in certain plants is a good illustration. It is ostensibly a quantitative feature, and the talls segregate clean from the dwarfs. But in various cases, *e.g.*, peas and sweet peas, the dwarfs are also a darker green. The dwarf of *Cam-*

[2] Richardson, C. W., *Jour. Gen.*, Vol. 3, p. 171 (1914).

panula persicifolia, especially (about 8 inches high), is a plant so strikingly different from the type (2–3 feet high), that it is sold as a species, "*C. nitida.*" The leaves of the dwarf are an intense, dark green.[3] This variety is, of course, a recessive and segregates without intermediates, yet, if the qualitative distinctions were less evident it might easily be classed as a variety in quantity. But the critical distinction is certainly qualitative and the great difference in size is consequential. Though in such cases segregation is complete, it may almost be said to be characteristic of purely quantitative distinctions that one or other of the original parental types fails to reappear in its extreme form after a cross. So usual is this feature in quantitative segregation that the phenomenon must have special significance.

Another fact is beginning to emerge which must contribute to the shaping of a conception of the physiological nature of segregation. We have learnt that groups or complexes of factors may segregate whole. To such a complex the distinction in sex is due, but in certain cases it may break up. The occurrence of a large spur in fertile hens (Leghorns for instance) must be regarded as due to the absence of that part of the sex-complex which in the normal inhibits the growing of the spur. In ordinary fowls the whole inhibiting group remains on the female side, but the spur-inhibiting element can evidently separate from the rest. On the other hand, when in the cocks of certain breeds (as occasionally in Wyandottes) there is no spur, we may perhaps conjecture that this element has been transferred to the male side.[4]

[3] The ovary projects in a curious way above the sepals so much that, were the plant not a *Campanula*, botanists would describe this ovary as superior.

[4] After much experiment the genetics of spur-development are still very obscure. In the course of a long series begun by crossing spurred Leghorn hens with a spurless Wyandotte cock (giving F_1 normal in both sexes), neither form has reappeared in F_2 with its original development. Very rarely a hen with minute spurs has appeared, and occasionally a cock with the spurs sensibly reduced. Nor from spurred hens $\times$ F_1♂ has anything approaching the original types been raised. Conceivably the detached element is able to join again with the test of the female complex.

The presence of the characteristically masculine comb and wattles in the male Sebright which is otherwise henny, shows that another factor similarly detachable governs their development.

To the breaking up of large compound factors the production of intermediate types, such as occur among the color-varieties of plants, is in all likelihood due. The sweet pea and the snapdragon have now an innumerable series of such color-forms which may be represented as having arisen by the disintegration of the various anthocyanins. That, at least, is the simplest way in which their origin can be conceived.

To the final result many qualifying elements contribute, and these may naturally be separate entities. But change in the amount of the same coloring material, and diminution in the mere extent of its distribution, are common features of these graduated series. As the cultural development of the species progresses, more and more of these quantitative intermediates appear, and are selected until a practically continuous series is produced. Although the interrelations of the whole series can be represented by a factorial scheme, the assumption that each of these factors had an aboriginal individuality appears gratuitous. In *Antirrhinum,* for instance, the ordinary self-colored flower segregates as a single unit from the white. But there are "Delila" forms having the "face" colored and the "throat" white. Another variety has the "lip" colored and the peripheral parts white and to this again there is an almost exact counterpart in which the peripheral areas are colored and the lip nearly white, and between these again there are further intergrades. Apart from factors modifying its quality, the color of the whole corolla, segregating as a single entity from the white, would without hesitation be represented as depending on a single factor. Subsequent experience that this entity can break up into an indefinite number of fractions is not evidence that the original representation was wrong. This reasoning applies to a great range of phenomena.

In view of the chromosome theory of linkage, it is therefore worth remarking that we do not find linkage especially frequent among these fractional factors. Have they, then, been distributed among different chromosomes? If in *Antirrhinum*, the color of the face and of the throat were lately parts of a single factor for the total flower-color, would not linkage between them be expected? Nevertheless, in cases of this sort, so far as I know, linkages have not been found with any special frequency.

The segregation of a group of differences—and presumably factors—in combination has lately been shown by Renner[5] to occur with extraordinary frequency in the Œnotheras, and this peculiarity is without doubt at the bottom of the difficulties which have beset the genetic analysis of these plants. The complexes are in several forms or species not borne equally by the two sexes of the same plant, and most of them are unable to exist in the homozygous state. These discoveries greatly elucidate the Œnothera problem. For instance, not only *Œn. lamarckiana*, but *biennis*, *muricata*, and others also, are not homozygous types, but heterozygotes of a special kind. Consequently, the production by them of "mutants" is not capable of the simple interpretation originally applied to them by de Vries. Renner suggests that the mutants arise owing to some interchange between the complexes which at present we can not investigate, but whatever be the exact manner of their origin we can not regard them as genuine examples of the production of novel forms by a homozygous type.

Before leaving this part of the subject, we may notice that the supposition that segregation is concerned solely with characters of a superficial or trivial nature has been long ago disproved. Baur's Antirrhinums, the study of which was continued by Lotsy, were an excellent demonstration to the contrary, for they provided many illustrations of segregation in features, the "specific value" of which no systematist would question. If further evi-

[5] *Zts. f. ind. Abstammungs- und Vererbungslehre*, XVIII, 1917, p. 121.

dence were needed, it may be found in the fine series of experiments lately published by Heribert-Nilsson[6] in *Salix*, which, contrary to the belief long ago entertained by Wichura, show that, when F_2 families are raised in adequate numbers, very striking segregation occurs in the species-crosses. Many geneticists are inclined to the view that segregable characters should be pictured as implanted on an irreducible base which is outside the scope of segregation, but no means have yet been devised for testing the reality of this conception.

2. *The Moment of Segregation*

The next question is to determine when in the various life-cycles segregation can occur. Obviously it is a phenomenon of cell-division. If we knew nothing of the genetics of plants we might confidently adopt the view which Morgan has so successfully developed, that normal segregation and redistribution happen exclusively in the process of reduction. Though unconvinced, I can not deny that linkage and crossing-over may well be represented provisionally, as effected during synapsis. The scheme previously offered by Punnett and myself as a diagrammatic plan capable of representing these phenomena is certainly far less attractive. There is evidence that in certain plants, *e.g.*, Matthiola, the composition of the families derived from single pods shows very great and perhaps irregular fluctuations, and the normal ratios for those families is only found by taking the average of many, but no sufficient statistical examination of such cases has yet been made. In some suitable case estimations of the offspring derived from individual anthers might be of value in this connection. Renner, by examining the starches of the pollen grains in Œnotheras, has lately made visible that dimorphism, of which we had previously genetic proof, and perhaps this novel and striking observation might be used for the purpose of mapping the distribution of such a character

[6] Lund's "Universitets Arsskrift, N.F.," Avd. 2, Vol. 14, November 28, 1918.

among the pollen grains. Meanwhile, it must be granted that no indication that gametic linkage results from somatic differentiation has yet been obtained.

When, however, we examine the view that linkage of factors is a consequence of their association in a chromosome, we must observe that there is no body of evidence that the number of linkage-systems agrees with that of the chromosomes, a primary postulate of Morgan's theory. *Drosophila* is the only example which has been adequately investigated. The cytological appearances are not readily consistent with the other postulate of Morgan's case, that crossing-over is effected by anastomosis of chromosomes and exchange of materials between them. In our present ignorance of the physical nature of the factors we are not obliged to assume that an actual transference of material is an essential condition for an exchange of properties; but since Morgan's suggestion is made in that form we are bound to notice how difficult it is to interpret the visible phenomena of cytology in accordance with that hypothesis. Without personal familiarity with cytology no one can have a confident opinion. I observe, however, that in his most recent publication on these subjects, E. B. Wilson[7] gives a very emphatic "counsel of caution," remarking that writers on genetics have taken too much for granted, and that for the present the genetic development of the chromosome theory has far outrun the cytological. To a layman the visible appearance of chromosomes is scarcely suggestive of the prodigious material heterogeneity demanded, and the general course of cytological evidence seems to indicate that the rôle of the chromosomes is passive rather than active. Though showing features of regularity, they are capable of very wide variations without transgressing the limits of viability, which could scarcely be the case were every detail in their organization critical. The appearance of chromosomes is not to me suggestive of strings of beads of extreme heterogeneity, but rather of strands of some more

[7] AMER. NAT., p. 210, May–June, 1920.

or less homogeneous substance; and in so far as numerical and geometrical order is exhibited by them, it would, in my opinion, be more proper to compare this regularity with that seen, for example, in drying mud or in the formation of prisms of basalt, than to attribute to it a more fundamental meaning.

Leaving these speculative considerations, and limiting our inquiry to the concrete question, at what moment in the cycle does genetic segregation occur, we reach a perfectly definite answer: that whatever future research may decide as to the occurrence of segregation in animals—which, for aught we know, may always be effected at the reduction division—there is no such limitation in plants. We are now thoroughly familiar with a large group of examples in which the genetic properties of the male and female cells of the same plant are quite different. In these, at all events, the reduction-division can not be the moment of the segregation by which these characters are distributed.

The first case detected was in *Matthiola,* where Miss Saunders' results proved that in the double-throwing singles the pollen carries exclusively doubleness, the eggs being mixed, some single and some double. A similar condition was shown to exist in regard to the cream and white plastids, respectively, the pollen grains bearing exclusively cream. De Vries observed a comparable arrangement among the Œnotheras, and Renner has lately shown that the phenomenon is widely spread in that group, thereby providing a consistent interpretation of much that was formerly obscure in the genetic behavior of these plants. In *Campanula carpatica* Miss Pellew proved that the pollen grains of the hermaphrodite form called *pelviformis* carry exclusively femaleness, and preponderantly white flower-color (the plant being heterozygous for blue). The case of Petunia investigated by Miss Saunders[8] is somewhat peculiar in the fact that in the heterozygous singles the male side carries the dominant singleness only, since in those in-

[8] *Jour. Gen.,* Vol. 1, p. 57 (1910).

stances to which the conception of dominance can be applied, it is the male which commonly carries the recessive. Segregation of these characters can not in plants so organized be supposed to take place later than the constitution of the male and female organs, and therefore the reduction division can not be the one critical moment. The suggestion has been made that germ-cells of the missing kinds may be in reality formed and perish before reaching a functional stage. As regards the Œnotheras, where shriveled pollen grains abound, this conjecture is very plausible and probably correct; but when, as in the other cases here quoted, the pollen grains are uniformly sound, the hypothesis is inapplicable and without evidential support. Moreover, even if it were true that certain classes of germ-cells perish in one or other of the sexes, that would hardly alleviate the difficulty, for this differential viability would remain to be accounted for, being itself a phenomenon of segregation.

Begonia Davisii[9] is another curious illustration in which segregation must occur even earlier. This plant is a wild, true-breeding species, with ordinary single flowers. All the pollen grains, however, carry doubleness, and used on the female flowers of doubles give offspring all double (single being the dominant). The pollen of this plant is as uniform and perfect as that of any species I have ever seen. We must therefore conclude that the segregation by which singleness separates from doubleness is effected not later than the formation of the rudiments of the male and female flowers. Cytological investigation may no doubt show that the distinctions between the genetic properties of the male and female are associated with visible nuclear differences, but I see no reason for anticipating that such differences must exist. Cells which differ in their genetic potentialities must of course differ in physical constitution, but there is no reason to suppose that this difference need be in any way dependent on chromosome structure.

[9] *Jour. Gen.*, VIII, 1919, p. 199.

As regards *Campanula carpatica "pelviformis"* and *Begonia Davisii,* experiment has shown that the peculiar kind of segregation which they exhibit may recur in their offspring. In the *Begonia,* if the female of *Davisii* be fertilized with pollen of an ordinary double tuberous *Begonia,* the doubleness so introduced stays on the male side just as the doubleness of its own male does, and a plant so bred has its pollen all double. But if the male of *Davisii* be used on the female of an ordinary single, there is no restriction of doubleness to either sex of the offspring. The peculiarity of *Davisii* must therefore be attributed to the special properties of its female side. The *Campanula* case is complex and has not yet been fully explored, but at least from the female side of *pelviformis* plants have been raised which retain the properties of the mother as regards the distribution of the white and blue colors.

We have at the John Innes Institution been lately investigating a simliar case in flax, which, though comparable, has some special features. A dwarf flax (*Linum usitatissimum*) of unknown origin, presumably a stray seedling of one of the varieties grown for oil, was fertilized with pollen from our tall fiber strain. Both parents breed true to the fully hermaphrodite condition, with anthers perfectly formed, and the F_1 plants were normal in this respect. F_2 consisted of hermaphrodites, and a recessive form with aborted anthers, generally contabescent and not dehiscing at all, but having occasionally a few grains of good pollen. The ratio was a normal 3:1. The recessive, having occasional grains of pollen, self-fertilized, gave similar plants with anthers wholly or almost wholly aborted. The normal F_2 hermaphrodites gave in F_3 families which showed that some of the F_2 plants were pure normals, others heterozygous in the ordinary way. But when the recessives were fertilized with pollen from three several varieties of tall fiber flax, only recessives were produced. These tall flaxes therefore are normally heterozygous for the recessive or "subfemale" condition, and this in segregation is perman-

ently relegated to the male side of the plant, while the female side takes the hermaphrodite factor. Segregation in regard to the same recessive may take place in one of two ways. It may be *unilateral,* as it is when in heterozygous association with the female of the tall flaxes, or it may be *ambilateral* and unrestricted to either sex when it is in association with the female of the oil flax. We must infer that the female halves of the two types differ in some critical respect which decides the manner of the segregation.

Unilaterality may also show itself as a difference in the closeness of linkage on the two sex-sides of the same plant, and no doubt this fact may have a bearing on the interpretation of the foregoing cases. The late R. P. Gregory discovered the first case of this, in *Primula sinensis.* He found that the linkage between magenta color and short style was closer in the eggs than in the pollen. Recent work on a larger scale has given 10.9:1 as the female linkage and 6.4:1 for the pollen. A similar difference has been also found for the linkage between green stigma and "reddish" stem (as opposed to dark red), the value being 29.8:1 for the eggs and 41.7:1 for the pollen. In both examples, individual families show wide fluctuations, and these values should for the present be regarded as approximate only. Whatever be their meaning, they show that some segregation has occurred in the formation of the two sets of sexual organs, such that the process of gametic differentiation is not the same in both.

Besides these examples of differentiation between the male and female sides, there are others proving that segregation may occur at other stages in somatic development. The most obvious examples are the variegated plants. I have discussed this subject elsewhere in connection with reversible periclinal "chimæras" of white over green which produce shoots having the white inclosed with the green.[10] To these must now be added the cases in which the plants arising from adventitious

[10] *Jour. Gen.,* Vol. 7, p. 93 (1919).

buds differ from the plants which produce them. I have described one of these examples in *Bouvardia*. The pinkish white "Bridesmaid" gives the red-flowered "Hogarth" from its root-cuttings. Three similar occurrences have been found in fancy Pelargoniums. The root-cuttings of a white-flowered variety, "Pearl," give a red-flowered form very like "Mme. Thibaut." "Mrs. Gordon," which is a full rose-pink, with whitish edges, gives from its root-cuttings flowers in which the two posterior petals are marked with dark red, not unlike the variety "Cardiff." A more striking case is that of "Escot," which gives from its roots plants with bright pinkish red marks, those of the original being purplish red. The most curious feature of this case lies in the increased size both of the plant and the flowers coming from the roots, and it is scarcely possible to see the petals of "Escot," which are characteristically rolled back, side by side with those of the root-form, which are not only larger, but also flat, without surmising that this rolling back is an expression of the greater size of the larger petal contained within the smaller, causing a want of correlation between the growth of the inner and outer tissues.

Buckling or crumpling of leaves through want of correlation was a conspicuous feature of some of Winkler's "graft-hybrids," made from *Solanum nigrum* and *S. lycopersicum,* when the larger tomato was inclosed within the smaller species. We have had a precisely similar example in a salmon-fringed Pelargonium bred by Mr. Jarman, of Chard. The leaves are obviously buckled, the petals are laciniated, and the female parts aborted, though the anthers are perfect. This male and deformed flower is proper to the outer tissues only; for on two occasions the plants have produced shoots with large flat leaves and normal hermaphrodite flowers with their petals entire. Obviously, this normal plant was inclosed within a skin of the fringed type.

In all these examples, a somatic segregation has occurred which determines the genetic potentialities. The

interpretation that they are *periclinal* chimæras is probably correct for the most part. The fringed *Pelargonium* is obviously of this nature. Nevertheless, the fact that a root-cutting consistently produces a certain type of plant which is not the original does not prove that the distribution is periclinal. Another possibility is well illustrated by the case of a variegated *Spiræa ulmifolia*, having the stem, petioles, and (basal) centers of the leaves without chlorophyll.[11] The growing point has the power of laying down green tissue in the lateral areas only, the internodal regions being albinotic. Root-cuttings from this form give albino plants which die after the development of two or three small leaves. Now in this case we can see the distribution of the green and white, respectively, and we recognize that the roots give albino plants because they belong wholly to the albinotic area. On similar lines it is possible to interpret the *Bouvardia* and other cases. The distribution of the two types in the same plant may be such that one is limited to the root and internodes, while the other is in the nodal structures.

That considerations of this kind are not fantastical is proved by the genetical phenomena seen in the case of "rogues" in culinary peas, which Miss Pellew and I have been investigating for a number of years.[12] The rogue is a peculiar, wild-looking plant, differing in various ways from the type, chiefly in having pointed leaflets. Crosses between it and the type give plants which in their lower parts are intermediate, though turning into rogues as they develop. The self-fertilized offspring of rogues and also of these F_1 plants are always rogues, and evidently the type-characters introduced

11 This is somewhat like the *Pelargonium* named by Messrs. Cannell "Freak of Nature," in which the chlorophyll has a closely similar distribution, and it, like the *Spiræa*, is sterile on both male and female sides. In this *Spiræa* I have never seen pollen, but very rarely a fruit is formed, which, no doubt, is due to an occasional development of a bud in the green area, an occurrence frequent in variegated plants. Whether these fruits contain viable seeds is not yet known.

12 *Roy. Soc. Proc.*, B, Vol. 91, p. 186 (1920).

from the type parent are left behind in the lower parts. Such a case may perhaps be compared with the condition seen in the variegated *Spiræa*, and we may fairly conjecture that if it were possible to raise root-cuttings from the F_1 peas, they would produce types.

A more gradual exclusion of the type-elements in the lower parts is seen in certain intermediates. These may scarcely differ from types in the lower parts, though changing to rogues, sometimes abruptly, sometimes gradually, as the series of flowering nodes is developed. Reciprocal crosses between the successive flowers of such plants and the flowers of types has shown that, together with the gradational change in the somatic structures, there is also a gradational change in the proportion of gametes bearing the rogue and type characters respectively. This proportion and the rapidity of the change differ on the male and female sides. Of the *egg cells* in the lower flowers, up to about the tenth flowering node, rather more than 50 per cent. carry the type-characters —or at least the non-pointed leaflets—but above this level the proportion of types declines. Of the *pollen* in the lowest two flowers only about 20 per cent. is type-bearing and the proportion diminishes rapidly in each successive flower above the level.

In all the examples given hitherto the segregation is in diploid tissues, but a comparable phenomenon has been proved by Collins to occur in the *haploid* axis of a moss (*Funaria*). In a diœcious moss, as the Marchals have shown, sex-segregation occurs at spore-formation, the division in which reduction is effected. This, of course, agrees with cytological expectation, though, so far as I know, the details have not been observed. But from the leaves of mosses placed in nutrient fluids new plants may be raised without great difficulty, and Collins found that the (perigonial) leaves surrounding the male organ thus propagated, produce *exclusively male* axes.[13] He has since raised similar cultures from the (peri-

[13] *Jour. Gen.*, Vol. 8, p. 145 (1918–19).

chætial) leaves surrounding the female organ, and, as related in his recent paper (Genetics of Sex in *Funaria hygrometer*), from them monœcious plants resulted. The proof is thus complete that in a haploid tissue a segregation of sex can occur.

The inference may be drawn that the factors for other characters may similarly be liable to segregate in the haploid state. In this connection I may mention a case which, though as yet obscure, perhaps fulfils this expectation. In botanic gardens a variegated maidenhair fern (*Adiantum capillus-Veneris*) is grown which has wedges of white tissue irregularly distributed in the segments. This plant produces spores freely,[14] and these give rise to prothallia which in several cultures raised here have always been entirely green. But when ferns arise from these green prothallia by the sexual process they are of three kinds, green, white, and variegated like the parent plants. The fact that the prothallia should be all green is entirely unexpected and creates a distinct problem, but it is evident that segregation must occur either in some of the cell-divisions by which the prothallia proliferate, or in those by which the gametes are formed; in either case in haploid tissue. This segregation is essentially different from that by which the differentiation of organs, such as the archegonia and antheridia, is accomplished, inasmuch as it relates to elements determining the characters of the next generation.

From the evidence given it is clear that in a wide view of living things segregation can not be exclusively a property of the reduction-division, and for the present it should be regarded as a possibility which may occur at any division in the life-cycle.

[14] I have not satisfied myself that spores are produced in sori on the white areas.

FACTORS IN THE RESISTANCE OF GUINEA PIGS TO TUBERCULOSIS, WITH ESPECIAL REGARD TO INBREEDING AND HEREDITY

DR. SEWALL WRIGHT, S.D., AND DR. PAUL A. LEWIS, M.D.

Bureau of Animal Industry, U. S. Department of Agriculture, Washington, D. C., and The Henry Phipps Institute, The University of Pennsylvania, Philadelphia, Pa.

In the pre-bacteriologic era physicians were quite generally of the opinion that heredity played a large part in resistance or susceptibility to tuberculosis. Following Koch's demonstration of the part played by the *Bacillus tuberculosis* in the etiology of the disease it was recognized that the hereditary influences might be only apparent; the disease being established in any family there was evidently an immeasurably increased chance of interfamilial infection; and as a consequence of this uncertainty of interpretation it has become quite customary to regard hereditary influences, properly considered, as a negligible factor.

Pearson[1, 2] and Goring[3] compared the correlation between parent and offspring, in incidence of the disease, with that between husband and wife. The effects of unfavorable conditions and the chances of infection might be about as great in one case as in the other, while heredity would be a common factor only in the first case. Pearson dealt with upper class families of which one member was being treated in a certain sanitarium. The correlation between parent and offspring came out about .50, in the usual scale, in which 1.00 is perfect correla-

[1] Pearson, Karl, 1907, "A First Study of the Statistics of Pulmonary Tuberculosis." Dulau & Co., London. 26 pages.

[2] Pearson, Karl, 1912, "Tuberculosis, Heredity and Environment." Dulau & Co., London. 46 pages.

[3] Goring, Charles, 1909, "On the Inheritance of the Diatheses of Phthisis and Insanity." Dulau & Co., London. 28 pages.

tion, after making certain assumptions in regard to the frequency of the disease in the whole population, and making allowance for the age of the children, many of whom had not reached the age of greatest danger. This correlation is about the same as that between parent and offspring in characters such as height, weight, eye color, etc., which are largely or wholly determined by heredity. The correlation between husband and wife was only .24 and even much of this, the author found reason for attributing to assortative marriage.

Goring's study was made with the families of British convicts. He also found a correlation of about .50 between parent and offspring but no correlation (— .01) between husband and wife. The only assumption that had to be made in this case was a correction for the age of the children.

We are acquainted with no experimental work bearing on this question.

Material

The present paper describes the results of experiments on certain inbred and crossbred stocks of guinea pigs. All of the animals used were a by-product of experiments on the effects of inbreeding which have been carried on by the Bureau of Animal Industry of the U. S. Department of Agriculture since 1906. One of the authors (Wright) has been in charge of these experiments since 1915. A detailed report on the results is soon to be published. A brief summary will suffice here.

Twenty-three families of guinea pigs were maintained for a number of years by mating exclusively brother with sister among the descendants of twenty-three original pairs. In sixteen cases (including families 2 and 13 of those tested for resistance to tuberculosis) both of the original parents came from a stock which had already been maintained for twelve years without the infusion of fresh blood, by the Experiment Station of the Bureau of Animal Industry. In the remaining families, including families 32, 35 and 39, the original females came from

the above mentioned stock, while the original males were purchased from a local dealer.

In 1911 a number of animals were selected from the stock of the Experiment Station to start a control experiment. The mating of even second cousins has been avoided in this stock, which has been called Experiment *B*.

Eighteen of the inbred families were still on hand in 1916, having then on the average about eleven generations of brother-sister matings back of them. At this time most of the families were disposed of in order to make room for cross-breeding experiments and to obtain larger numbers from the five families which it was decided to retain. These families, numbers 2, 13, 32, 35 and 39, are the ones which have been tested for resistance to tuberculosis. They were retained in part because they already occupied many pens, but largely because of the possession of contrasting characteristics in size, fertility and coat pattern. Each of these families is at present very homogeneous in heredity. Family 2 is descended wholly from one mating in the sixth generation of inbreeding. Families 13, 32, 35 and 39 come from single matings in the seventh, eleventh, twelfth and eighth generations respectively.

The total number of animals involved in the experiment on inbreeding and cross-breeding has been about 30,000.

THE EFFECTS OF INBREEDING ON VIGOR

It is noteworthy that there has been no very obvious decline in vigor, although the families are now on the average in the fourteenth generation of brother-sister mating and one of the most vigorous (No. 35) has reached the twenty-first generation.

There has, however, been some decline in vigor in all respects which have been studied. The decline is most marked in fertility, including both frequency and size of litters. It has been so great in this respect that it would have to be recognized even though the decline in

other respects were assumed to be due wholly to less favorable environmental conditions.

That there has been a real genetic decline in the inbred stock in all elements of vigor is shown by comparison with the control stock *B*, which has been superior in every respect. Still better evidence has been obtained by comparison of the inbreds with the young from crosses between the different families raised at the same time and under the same conditions.

The Effects of Crossing

In interpreting the effects of crossing, the characteristics which depend on the hereditary make-up of the young must be distinguished from those which depend on the dam or sire. In studying these questions, inbred females have been mated with inbred males of another family (experiment *CO*) and with crossbred males (experiment *CA*). Crossbred females have been mated with brothers (experiment *CI*), unrelated crossbred males (experiment *CC*) and inbred males of an unrelated family (experiment *AC*).

Size of litter appears to depend wholly on the dam. There is little or no improvement in the experiments in which the dam is inbred (*CO*, *CA*). There is, however, a marked increase, 10–30 per cent. depending on conditions, in the litters produced by crossbred females (*CI*, *CC*, *AC*).

The record of an experiment in frequency of litters depends on the age of maturity as well as on the regularity thereafter. Males mature considerably later than females, so that the age of maturity of the male is the controlling factor in this respect in matings, made as in the present experiments, between immature animals. The frequency of litters after maturity appears to depend largely on the dam. There is no improvement in the record of the first cross (*CO*) over the inbreds. There is, however, marked improvement in the other experiments in which either the sire or dam or both are cross-

bred. Crossbred males mature earlier and the crossbred females have litters more regularly than inbreds.

The percentage of the young born alive depends almost wholly on the dam. There is little or no improvement in experiments *CO* and *CA*, but an increase of 6 to 8 per cent. where the dam is crossbred. The percentage which are raised to 33 days of age of the young born alive depends both on the dam and on the heredity of the young. There is a marked increase, 9 to 12 per cent., in all of the crossbreeding experiments mentioned above.

Somewhat similarly, birthweight depends largely on the dam, while the gains between birth and 33 days depend to a considerable extent, though far from wholly, on the heredity of the young. Guinea pigs become independent of the dam at a very early age. There is an increase of 2 or 3 per cent. in experiments *CO* and *CA*, but one of about 7 to 10 per cent. where the dam as well as the young are crossbred. In the gain between birth and 33 days, there is an improvement of about 16 per cent. in the first cross, which is somewhat increased in the young produced by crossbred dams. There is an increase of 15 to 20 per cent. in the adult weight in the first cross (*CO*). This is maintained in the young produced by matings of unrelated crossbreds (*CC*), but half of it is lost where the parents, though crossbred, are brother and sister (*CI*). The influence of the dam does not appear to extend to the adult weight.

A loss in the improvement brought about by the crossing becomes apparent in the second generation of inbreeding following a cross (experiment *C*2) in those cases in which it is not apparent in the first generation.

COMPARISON OF DIFFERENT FAMILIES

A comparison of the different inbred families with each other has revealed persistent differences in color, pattern, tendency toward polydactylism, tendency toward production of monsters, mortality among the young, weight and both elements of fertility. It was found that

the differences in these respects could not be interpreted merely as differences in general vigor. Vigor above the average in one respect was as likely as not to be found associated with a subnormal record in another respect, the correlation between the records of the families in two respects coming out in most cases substantially zero. Examples of these family differences will be brought out later in connection with the effects of inoculation with tuberculosis.

Explanation of the Results of Inbreeding and Crossing

These results harmonize well, on the whole, with those found by other investigators. It is believed that they can be explained as consequences of the current theory of heredity without recourse to the rather mystical ideals which once prevailed in regard to inbreeding. There appear to be independently inherited factors which affect frequency and size of litter, ability to bear the young successfully, vitality and growth as well as for color, pattern and the other characters in which the families differ. There seem to be surprisingly few factors which act on all of these characteristics. The concept, hereditary vigor, thus becomes merely an expression for the sum of a number of independently inherited qualities and not an entity.

The factors which cause reduced vigor in any respect appear to be in general recessive. The primary effect of inbreeding is to render homozygous a random group of the factors present in the original stock. Some combination of factors, good, bad and indifferent, thus becomes fixed in each inbred line. As the recessive factors, tending toward lack of vigor, are as likely to become fixed as the dominant ones there is on the average a decline in vigor in each respect. Moreover, owing to the likelihood that many factors for vigor will be linked genetically with factors causing weakness, it is to be expected that vigor in all respects will be found in very few lines, even where there is careful selection. In the pres-

ent case, there was very little conscious selection but a considerable amount of natural selection was, of course, unavoidable.

On crossing two inbred lines, each, as a rule, supplies some of the dominant factors lacking in the other, with the consequence that there is increased vigor in so far as the character in question depends on the heredity of the animal itself. In the next generation if brother-sister matings are made, there should be a decline as compared with the first generation in characters which depend wholly on the animal itself. The decline from this cause may, however, be balanced or more than balanced by the improvement due to the influence of the crossbred dam.

EXPERIMENTS ON RESISTANCE TO TUBERCULOSIS

Since April, 1919, all surplus individuals in five inbred families (2, 13, 32, 35 and 39) and a random selection from certain crossbreeding experiments (*CO*, *CA*, *AC*, and *B*) have been shipped from Washington to Philadelphia to be tested for resistance to tuberculosis. The animals were shipped in lots of 30 to 60 every two or three weeks. They were from 5 to 8 weeks old at the time of shipping. They usually arrived in good condition but with some loss in weight. The first two lots were not tested because of heavy mortality before inoculation due to feed conditions. Lot 6 was not tested because of heavy mortality due to excessive heat during shipment. Data on the shipment and inoculation are presented in Table I. The deaths within 15 days after inoculation are probably not attributable to tuberculosis and are not considered in the later work. The experiments were considered closed when all but two or three of the animals had died. The last column gives the number of days after inoculation at this time. The next to the last column gives the day by which 50 per cent. of those which passed 15 days had died. It agrees fairly well with the average number of days calculated on the assumption that the few survivors of the experiment died on the day following that given in the last column.

The cultures used were of the human type of tuberculosis with the exception of lot 3 in which the type was bovine.

TABLE I

Lot	Date Shipped	Date Inoculated	Inoculation	No. Died Before 15 Days	No. Survived 15 Days	Av. No. of Days Lived	Day to which 50 % Survived	Day Exp. Closed
3	May 4	June 2, 1919	1/10 mg. culture 07.8 intraperitoneal	2	32	43.9	44	62
4	June 4	" 10, "	1/5 " 465 "	5	30	25.9	21	40
5	" 18	July 3, "	1/10 " 465 "	7	57	24.0	24	31
7	July 17	Aug. 25 "	1/5 " DG "	3	37	25.6	24	43
8	" 30	" "	" " " "	1	28	25.2	24	38
9	Aug. 14	" "	" " " "	3	44	25.3	24	39
10	" 28	Oct. 24 "	" " " subcutaneous	6	46	49.7	47	84
11	Sept. 11	" 25 "	" " " "	3	31	54.2	54	83
12	" 25	" " "	" " " "	2	30	49.5	46	83
13	Oct. 9	" " "	" " " "	3	42	45.2	40	83
14	" 23	" " "	" " " "	2	35	42.3	39	83

Lots 7, 8 and 9 were given the same inoculation on the same day. The results are so similar, in spite of the differences in the age and weight of the animals, that they can be dealt with satisfactorily as one experiment. Lots 10 to 14 were all given the same inoculation on October 24 and 25 and can also be dealt with as one experiment. The same quantity of the culture, inoculated intraperitoneally in lots 7 to 9, was inoculated subcutaneously in lots 10 to 14, with the consequence that the average length of life was much greater in the latter group of lots. Lots 3, 4, and 5 were inoculated separately with different cultures or different amounts of the same culture. The numbers in each case are rather small for separate treatment. The mortality curves for lots 4 and 5 are not, however, very different. That for lot 3 can be made similar by multiplying the days of survival after inoculation by 3/5. These three lots have been combined in this way for comparison with the later and more satisfactory experiments.

SEX AS A FACTOR IN RESISTANCE

In attempting to analyze the difference in the length of life following inoculation, we will consider first a number of possible factors other than heredity. It will be convenient to start with sex. Chart 1 shows the per-

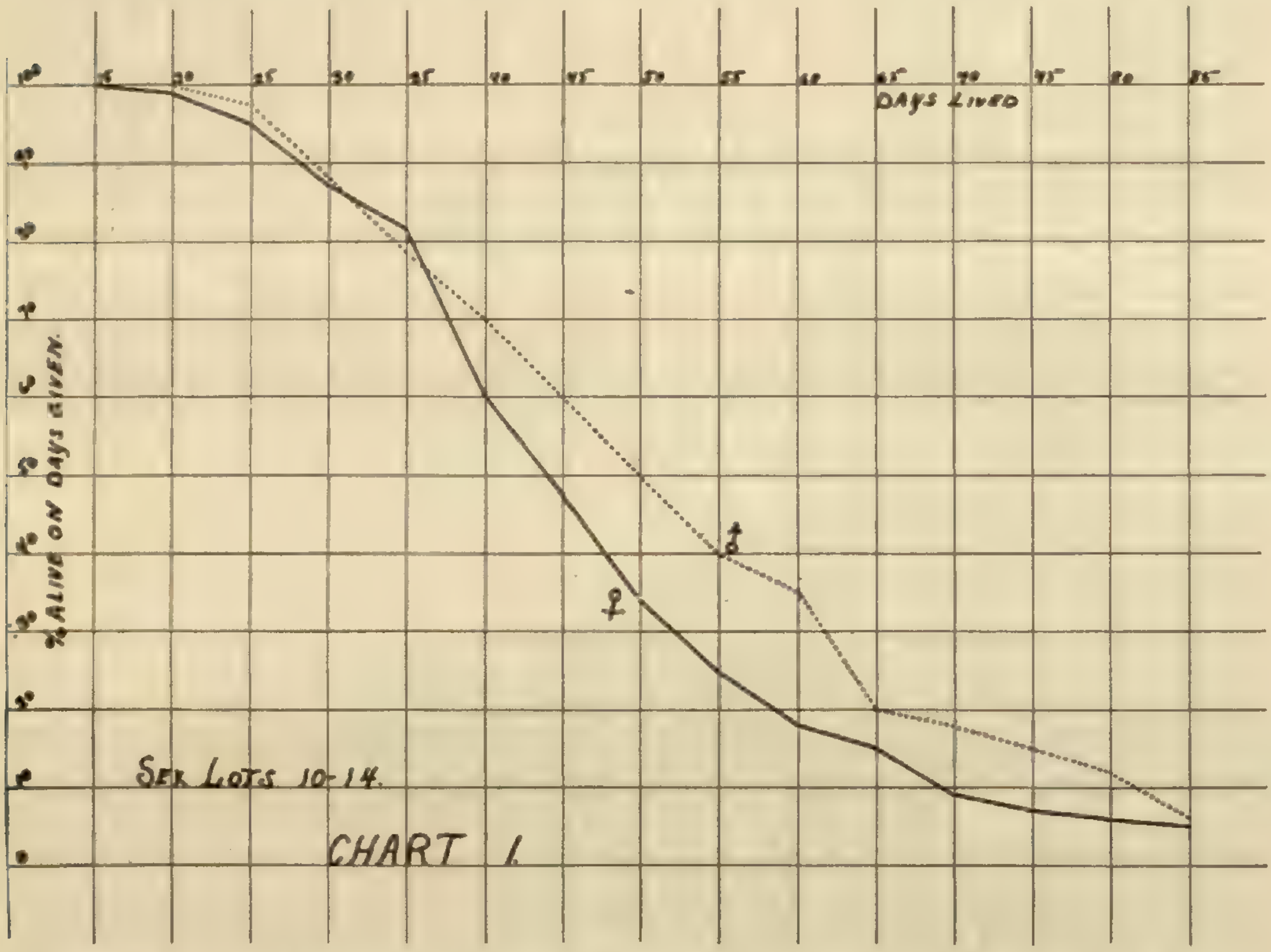

CHART 1. The mortality curves of the males and females of lots 10 to 14. This and the later charts give the percentage alive at the beginning of each five-day period starting with 100 per cent. on the fifteenth day after inoculation.

centage of the males and females alive at the beginning of each day in lots 10 to 14, starting with 100 per cent. on the fifteenth day after inoculation. The males have some advantage in lots 10–14, but it is too small to be of more than doubtful significance. In lots 3 to 9, moreover, the two mortality curves keep crossing each other in such a way as to indicate that sex makes no appreciable difference in resistance.

THE AGE OF THE DAM AS A FACTOR

Some rather dubious indications of a relation between age of mother and susceptibility of the offspring to tuberculosis were found by Pearson among human beings.

The guinea pigs in lots 10–14 were divided into two groups, those containing at least 25 per cent. of the blood of family 35 and the remainder. Each of these groups was classified by birth rank with results given in Table II.

TABLE II

AVERAGE NUMBER OF DAYS SURVIVAL AFTER INOCULATION AMONG THE YOUNG IN DIFFERENT BIRTH RANKS, LOTS 10–14

Order of Litter	Animals with Blood of Family		Miscellaneous	
	No.	Av.	No.	Av.
1	16	58.9	17	35.6
2	21	60.3	29	43.1
3	7	70.6	23	43.3
4	12	63.3	26	39.8
5	4	60.8	6	35.8
6			11	45.9
7			5	37.1
8			3	33.3
8 +			4	30.8

These figures suggest an increase in resistance of the young born in litters up to the third and a decline in resistance in young born of aged dams. They must be confirmed by larger numbers, however, before much significance can be attached to them.

THE RELATION OF AGE, WEIGHT AND RATE OF GAIN TO RESISTANCE

Mere bulk must be considered as likely to be a factor in resistance to tuberculosis. Other things being equal, one would expect to find that the same inoculation given to a St. Bernard and to a toy spaniel would have a more disastrous effect on the latter. In lots 10–14, some of the guinea pigs were over three times as heavy as others at the time of inoculation (variations between 120 and 440 grams).

The rate of gain is a good indication of the condition of the animals and is thus another factor which one would expect to find correlated with resistance to disease. In this connection, we have for study the birth-weight, weight at 33 days and the rate of gain between 33 days

and inoculation. The birthweight varies between 40 and 120 grams, the weight at 33 days between 90 and 360 grams, and as to rate of gain after 33 days, some lost weight while others gained over 3 grams a day.

Age is a factor which must be considered apart from weight and rate of gain. In lots 10–14, there were varia-

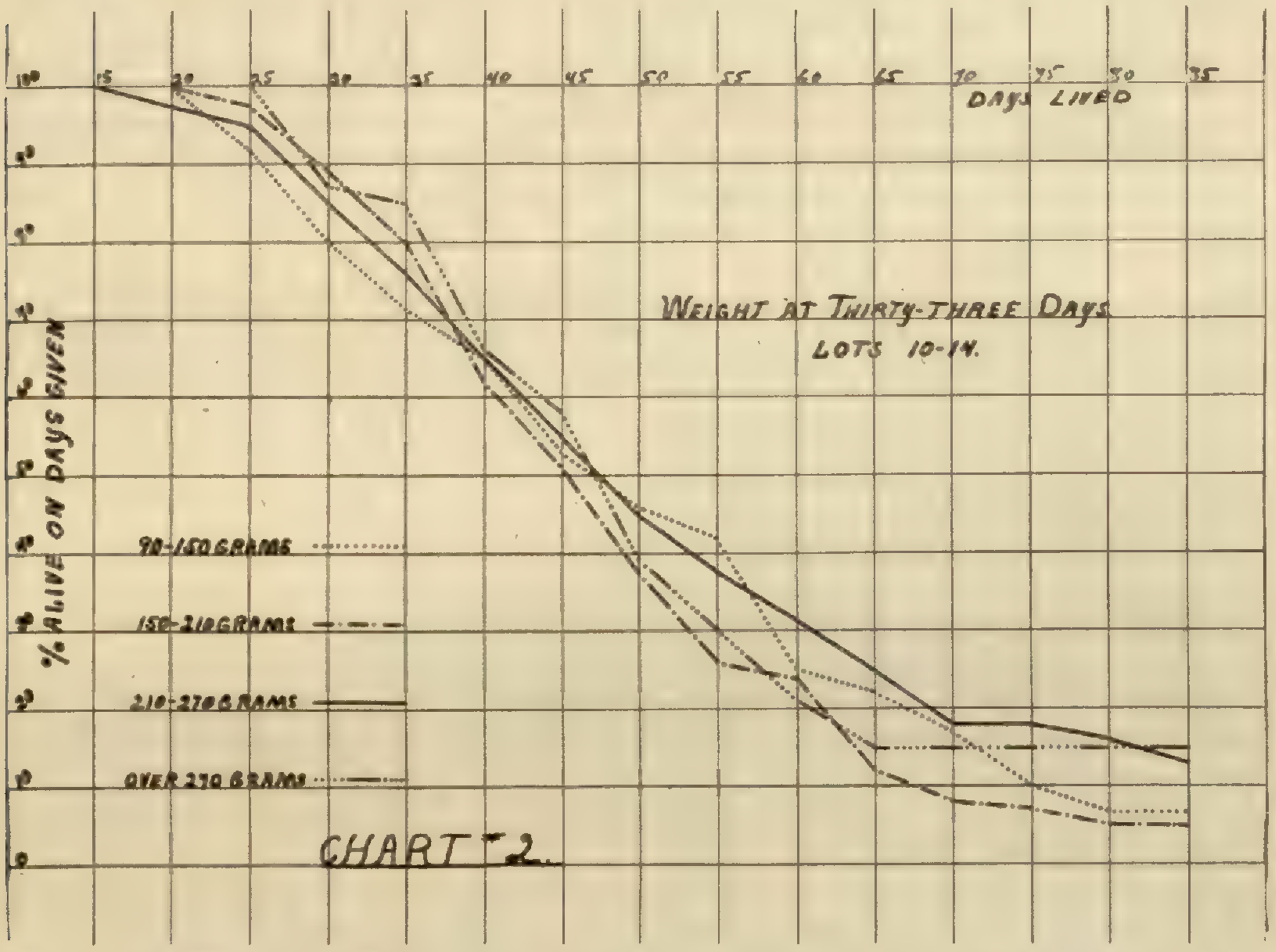

CHART 2. The mortality curves of the guinea-pigs of lots 10–14, grouped according to weight at weaning.

tions between 35 and 105 days at the time of inoculation. In lots 7–9 the variation was between 45 and 85 days.

The correlation between the length of life after inoculation and each of these factors, including also size of litter, is given in Table III for lots 7–9 and lots 10–14. All of these correlations were calculated by the usual product

TABLE III

Factors Correlated	Lots 7, 8, 9	Lots 10–14
Days lived—Size of litter	+ .090 ± .064	+ .029 ± .050
—Birth weight	— .044 ± .064	+ .059 ± .050
—Weight at 33 days	+ .022 ± .065	+ .056 ± .050
—Rate of gain (33-inoc.)	+ .120 ± .064	+ .223 ± .047
—Age at inoculation	+ .025 ± .065	+ .180 ± .048
—Weight at inoculation	+ .092 ± .064	+ .219 ± .047

moment method. Perfect positive correlation is +1, perfect negative correlation is —1, while 0 indicates the absence of correlation.

Inspection of these figures reveals that all of them are surprisingly low. In lots 7–9, the factor most closely correlated with length of life is the rate of gain between weaning (33 days) and inoculation, and even this is not certainly significant in view of its probable error (+.120 ± .064). Larger correlations are to be expected in lots 10–14, owing to the greater heterogeneity in age and weight, but even here the correlations are all small. Size of litter, birthweight and weight at 33 days are again of no value as indicators of resistance to tuberculosis. There, are, however, significant correlations between length of life and rate of gain (+.223 ± .047), age (+.180 ± .048) and weight of inoculation (+.219 ± .047).

The degree to which variation is determined by a given factor is measured by the square of the coefficient of correlation. On this basis, only 5 per cent. of the variation in length of life (*i.e.*, 5 per cent. of its mean square deviation) is determined by the most important of the above factors. The degree of determination by all of these factors combined can not be found by merely adding the squares of the correlations, because the factors are not independent of each other. For example, rate of gain is an element in determining the weight at inoculation. Any effect which it has on resistance should be reflected in a correlation between weight and length of life as well as between rate of gain and length of life. The correlations among the more important of these factors are given in Table IV.

TABLE IV

		Lots 7, 8, 9	Lots 10–14
Weight at inoculation	—Rate of gain.....	+ .357 ± .056	+ .553 ± .035
	—Age	+ .420 ± .054	+ .742 ± .022
	—Weight at 33 days.	+ .780 ± .025	+ .670 ± .027
Rate of gain	—Age	+ .323 ± .058	+ .592 ± .032
	—Weight at 33 days.	— .153 ± .063	— .040 ± .050
Weight at 33 days	—Age	— .012 ± .065	+ .160 ± .048

Weight at inoculation is of course completely determined by the weight at 33 days and the rate of gain and age at inoculation. The correlations between weight at inoculation and these three factors are accordingly high. They are rather different in the two groups of experiments, but this is to be expected. There was much less

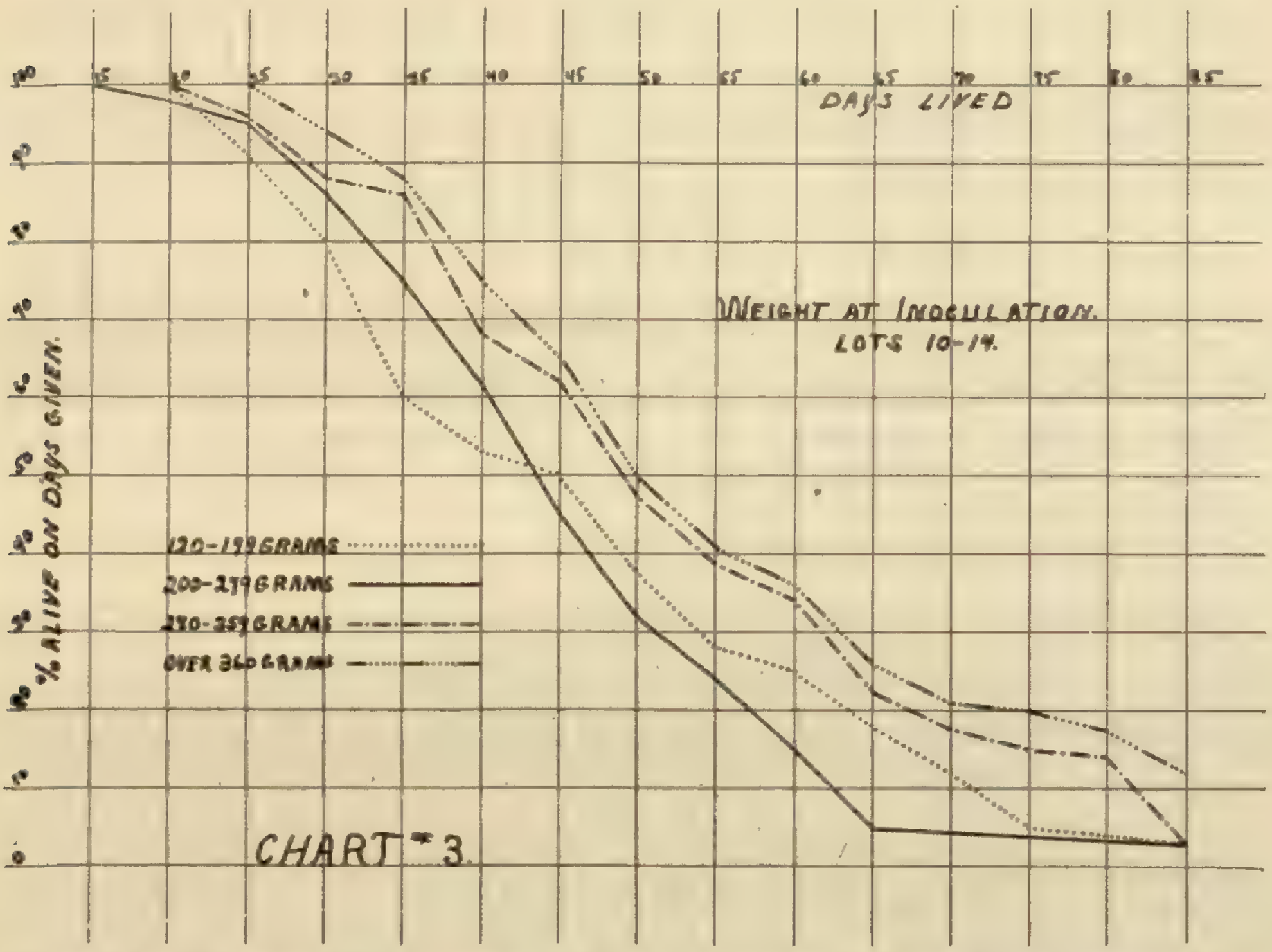

CHART 3. The mortality curves of the guinea-pigs of lots 10-14, grouped according to weight at inoculation.

variation in age in lots 7–9 than in lots 10–14 and variation in age accordingly plays a less important part in determining the variations in weight than in the latter case. The rather high correlation between age and rate of gain is due to the fact that nearly all of the guinea pigs suffered a loss in weight following shipment at a little over 33 days of age. Thus the older they were at inoculation, the greater the time in which they had to recover from the effects of shipping. The rate of gain before 33 days is generally an indication of the probable later rate of growth. In the present case, however, the slight negative correlation between the rates of gain before and after 33 days seems to indicate that shipping delayed

more the growth of those guinea pigs which had been gaining rapidly than of those which had more growth to make up. The small positive correlation between weight at 33 days and age at inoculation reflects a decline in the condition of the stock, due probably to the use of an inferior quality of hay, which took place during the sum-

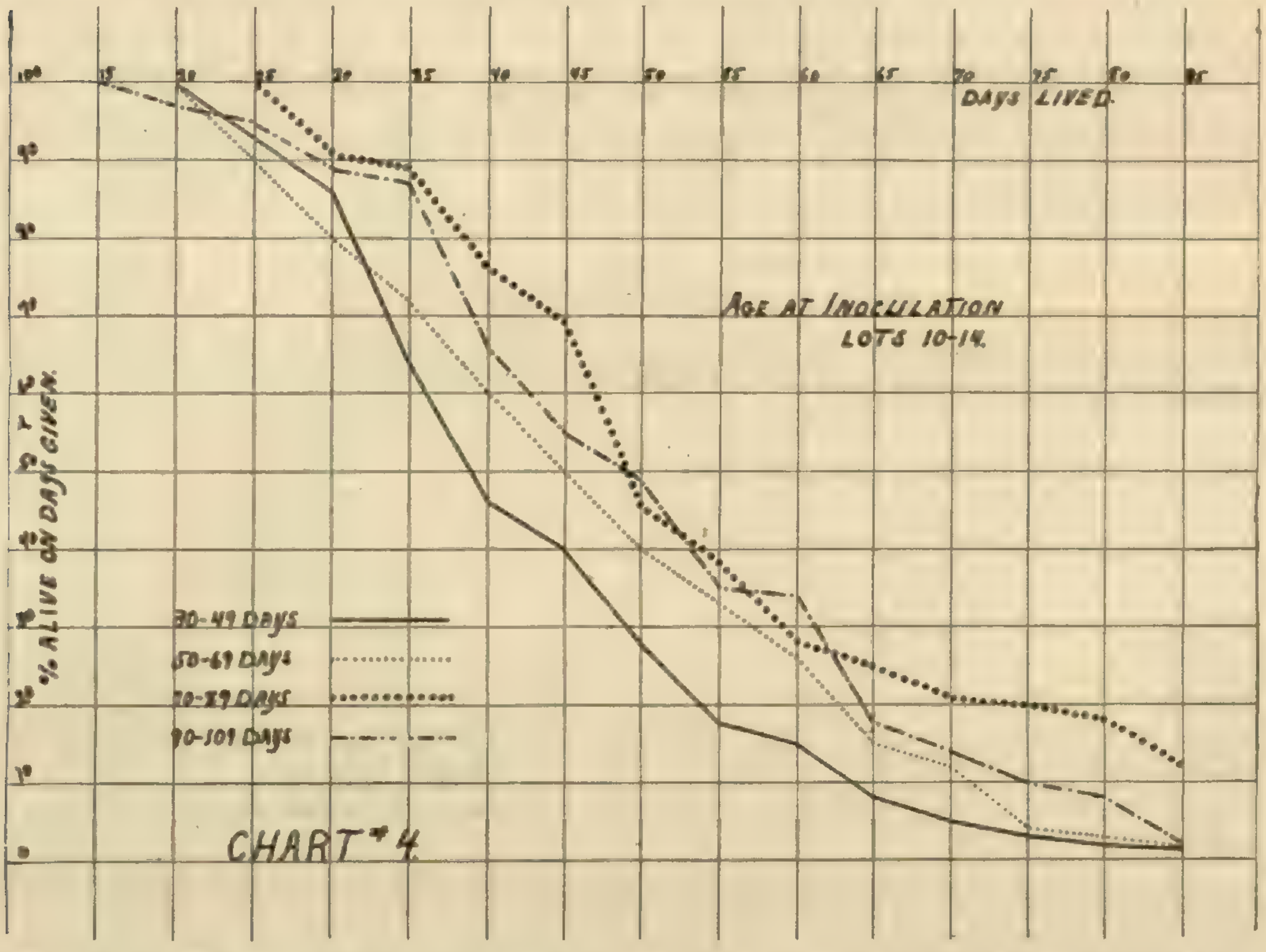

CHART 4. The mortality curves of the guinea-pigs of lots 10–14, grouped according to age at inoculation.

mer of 1919, especially during the time in which lots 10–14 were being raised.

With the help of these correlations, we can calculate the degree to which variation in length of life is determined by each factor separately and by all of them combined. For the last purpose, Pearson's coefficient of multiple correlation can be used. This coefficient comes out + .136 in lots 7–9 and + .251 in lots 10–14 for the correlation between length of life and rate of gain after 33 days, age and weight at inoculation combined. The degree of determination is measured by the square of this coefficient. These three most important factors combined, therefore, determine less than 2 per cent. of

the variation in length of life in lots 7–9 and less than 7 per cent. in lots 10–14. The separate contributions of the various factors can be calculated with results given in the following table.

TABLE V

DEGREE OF DETERMINATION OF DAYS LIVED AFTER INOCULATIION BY AGE, WEIGHT AND RATE OF GAIN

Factors	Lots 7–9	Lots 10–14
Direct effect of age	.0015	.0005
weight	.0049	.0229
rate of gain	.0116	.0233
age and weight	—.0023	—.0051
age and rate of gain ...	—.0027	—.0041
weight and rate of gain.	+.0054	+.0256
Total degree of determination	.0184	.0631

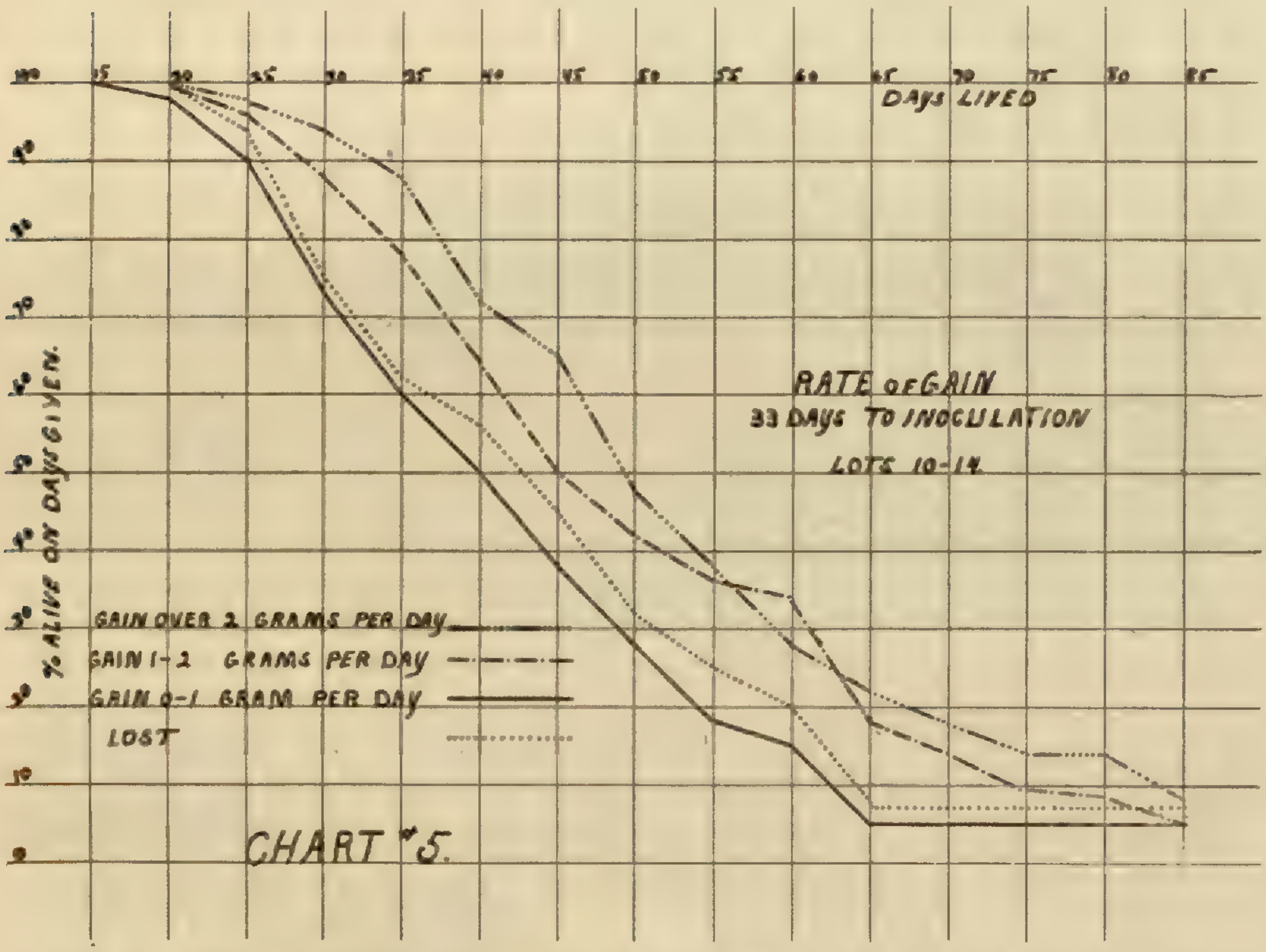

CHART 5. The mortality curves of the guinea-pigs of lots 10–14, grouped according to the rate of gain between weaning and inoculation.

While these figures can not be trusted in detail, owing to the large size of the probable errors of the correlations on which they are based, they indicate that age within the limits of one to three months has virtually no direct effect whatever on resistance to tuberculosis. There seems to be a slight direct effect of absolute weight

and a slightly greater direct effect of rate of gain, presumably as a measure of the condition of health.

NOTE

It may be of interest to readers who are not familiar with this method of analysis to compare the degrees of determination shown in Table V with those in a case in which we know a priori that there is complete determination of one variable by a number of others. The data for such a case have already been presented. The weight at inoculation is completely determined by weight at 33 days and the product of the rate of gain and interval between 33 days and inoculation. The coefficient of multiple correlation between weight at inoculation and these three factors combined comes out .9884 in lots 7–9 and .9628 in lots 10–14. The degrees of determination are as follows:

TABLE VI

Factors	Lots 7–9	Lots 10–14
Direct effect of weight at 33 days	.7106	.3689
rate of gain	.1501	.0907
age	.0930	.2177
Joint effect of weight at 33 days and rate of gain.	—.0500	—.0073
weight at 33 days and age	—.0031	.0907
rate of gain and age	.0763	.1663
	.9769	.9270

The departure of the multiple correlations and the sums of the degrees of determination from unity are of the order usually met in such cases. This method of analysis applies strictly only where the effects of the factors are combined by addition and the correlations are linear, conditions which are not perfectly met in the present case.

The facts brought out by the method of correlation are presented graphically in Charts 2, 3, 4 and 5. These charts show the decline in numbers, on the basis of 100 per cent. alive on the 15th day after inoculation among groups of guinea pigs of lots 10–14, classified by weight at 33 days, weight at inoculation, and rate of gain.

As a result of the foregoing considerations, it must be concluded that the apparent condition of a guinea pig at the time of inoculation and a knowledge of its past history give exceedingly little indication as to its probable length of life after inoculation. Over 98 per cent. of the variation in length of life in lots 7–9 and over 93 per cent. in lots 10–14 is caused by factors other than those discussed. This leads us to a consideration of hered-

itary differences as one of the possible causes of this variation.

HEREDITARY DIFFERENCES IN RESISTANCE

The average length of life in each inbred family and crossbreeding experiment in lots 3–5, 7–9 and 10–14 is given in Tables VII. VIII and IX together with other

TABLE VII

AVERAGES OF THE CHARACTERISTICS OF THE GUINEA PIGS TESTED FOR RESISTANCE TO TUBERCULOSIS IN LOTS 3, 4 AND 5.

The days lived in lot 3 are multiplied by 3/5 to make the average about the same as in lots 4 and 5.

Family or Experiment	Number Tested	Size of Litter	Birth Weight	Wt. at 33 Days	Wt. at Inoculation	Age at Inoculation	Av. Days Lived
2	28	2.85	73.3	201.1	209.8	54.6	23.1
13	15	3.13	83.4	232.7	227.7	49.7	20.3
32	2	2.50	80.5	256.0	215.0	53.0	21.0
35	9	2.56	86.5	252.0	236.7	50.5	25.7
39	1	4.00	77.0	157.0	140.0	49.0	16.0
CO	10	2.70	82.1	236.5	243.5	51.5	26.0
CA	24	3.08	85.5	247.4	247.7	52.0	25.7
AC	21	3.76	77.8	230.2	223.6	51.3	27.4
B	9	2.33	101.2	277.9	280.6	54.2	25.8
Inbred	55	2.89	78.5	219.2	218.0	52.4	22.6
Crossbred	64	3.14	84.6	244.4	243.7	52.0	26.3

TABLE VIII

AVERAGES OF THE CHARACTERISTICS OF THE GUINEA PIGS TESTED FOR RESISTANCE TO TUBERCULOSIS IN LOTS 7, 8 AND 9

Lots 7, 8, 9	No.	Size of Litter	Birth Weight	Weight at 33 Days	Weight at Inoculation	Age at Inoculation	Av. Days Lived
2	13	2.46	80.2	197.1	243.1	64.2	22.8
13	16	2.81	91.0	257.1	308.1	63.6	23.9
32	10	2.40	86.6	224.4	273.0	71.8	20.6
35	9	2.56	85.0	242.5	282.2	61.2	27.1
CO	13	3.00	80.2	226.2	286.2	67.8	31.5
CA	14	2.93	80.1	213.1	251.4	58.1	24.0
AC	21	3.38	74.6	213.0	257.6	61.9	25.6
B	13	3.38	83.7	249.5	320.0	69.2	24.8
Inbred	48	2.58	86.0	231.3	278.3	65.0	23.5
Crossbred	61	3.20	79.0	223.6	275.6	63.8	26.8

data. The distribution of the deaths is given in Tables X, XI and XII. As already stated, most of the lots were brought to a close while a few animals were still living. This introduces an element of uncertainty into the aver-

ages, but the assumption that these survivors died on the following day is unfair only to the superior stocks.

TABLE IX

AVERAGES OF THE CHARACTERISTICS OF THE GUINEA PIGS TESTED FOR RESISTANCE TO TUBERCULOSIS IN LOTS 10–14

Lots 10–14	No.	Size of Litter	Birth Weight	Weight at 33 Days	Weight at Inoculation	Age at Inoculation	Days Lived
2.....	27	2.56	73.1	175.0	254.4	76.9	45.3
13.....	18	2.67	84.2	221.1	301.1	91.3	35.7
32.....	5	2.20	88.0	223.2	228.0	63.0	35.4
35.....	22	2.73	83.8	231.6	301.4	72.8	58.5
CO.....	26	2.81	82.8	210.9	274.6	66.8	52.7
CA.....	33	3.45	72.3	195.6	274.8	69.8	56.5
AC.....	31	3.26	75.3	213.7	273.9	68.1	45.9
B.....	22	3.09	79.5	220.1	280.9	67.7	40.0
Inbred.....	72	2.61	80.2	207.2	278.6	73.3	46.2
Crossbred....	112	3.18	77.0	209.0	275.7	68.2	49.4

TABLE X

THE NUMBER OF DAYS FROM INOCULATION TO DEATH IN EACH EXPERIMENT IN LOTS 3, 4 AND 5

The intervals in lot 3 are multiplied by 3/5 to make them equal on the average to those in lots 4 and 5. The deaths before 15 days are given in Table I. The animals in experiments *CO*, *CA* and *AC* are also classified by the amount of blood of family 35 at the bottom of the table. The last column includes two animals in lot 3 which survived at 61 days, one in lot 4 which survived at 42 days, and 3 in lot 5 which survived at 30 days.

	Experiment																	
	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	30+	Total
2....	1	2	1			4	5		2	1	2	1	2	5	2			28
13....			2		2	3	5	1	2									15
32....						1		1										2
35....					1			2		1			1	1	2		1	9
39....		1																1
B.....						1	1	1			2	2		1			1	9
CO...							1	2	1	1	3						2	10
CA...				1	3	1	3	1		1	1	1	2	2	3		5	24
AC...					2	1	1			1	1	4.	1	3	2		5	21
0 (35).					5	2	5	1	1	3	5	2	1	3	3		6	37
¼ (35).								1				1	1	1	1		1	6
½ (35).				1				1				2	1	1	1		5	12

THE EFFECT OF INBREEDING

In each case, the crossbreds (*CO*, *CA*, and *B*) lived a little longer on the average than the combined inbred families. This result is in line with the results of cross-

TABLE XI

THE NUMBER OF DAYS FROM INOCULATION TO DEATH IN EACH EXPERIMENT IN LOTS 7, 8 AND 9

	Experiment																										
	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	42+	Total
2.....		3	1		1		3	2	2											1								13
13.....				1	2	4	1	4		2				1	1													16
32.....	1	1	2	2	1			3																				10
35.....						1		2	3						1	1				1								9
B.....						1	1	5	4	1																1		13
CO.....							3		2		1				1		1					2	1			1	1	13
CA.....						2	3	2	7																			14
AC.....			1	2	2	3	2	3	1				1	1	2		1			1		1						21
0 (35).....			1	2		4	6	1	5		1		1		2		1											24
¼ (35).....					1	1	2	2	2					1	1													10
½ (35).....					1			2	3								1			1		3	1			1	1	14

TABLE XII

THE NUMBER OF DAYS FROM INOCULATION TO DEATH IN EACH EXPERIMENT IN LOTS 10 TO 14

	Experiment															
	15	20	25	30	35	40	45	50	55	60	65	70	75	80	82+	Total
2.....		3	3	2	4	3	2	1	2	3	1	1	1	1		27
13.....			4	6	4	1	1	1	1							18
32.....	1			1	1	1	1									5
35.....				1	3	2	2	2	2	2	1	1	1	2	3	22
B.....		1	4	1	6	4	2	2	1	1						22
CO.....		1	1	1	3	3	3	3	3	2	1	2			3	26
CA.....				2	2	1	6	4	3	8	2		1	2	2	33
AC.....		2	4	2	4	3	6	3		2	2			2	1	31
0 (35).....		3	5	4	8	5	10	6	4	6					1	52
¼ (35).....				1	1	1	3	1	1	1	1			1	2	13
½ (35).....						1	2	3	1	5	4	2	1	3	3	25

ing on vigor in other respects. In the present case, however, the difference does not appear very striking. In particular, the superiority of the control stock *B* over the inbreds seems very dubious. An earlier test of this question was made by Mr. E. H. Riley and Dr. E. C. Schroeder, of the Bureau of Animal Industry, when the inbred stock was in the sixth and seventh generations of inbreeding. They found that the inbred was distinctly inferior to the control stock in resistance. At that time, however, eighteen families were on hand. It is not unlikely that the four families tested in the present experiment are a selected lot in respect to resistance.

Returning to Tables VII, VIII and IX, there are no differences between experiments *CO* (parents unrelated inbreds), *CA* (sire crossbred, dam inbred) and *AC* (sire inbred, dam crossbred), which indicate an influence of the breeding of the dam on the resistance of the young. There are rather large differences, it is true, but these are not consistent and must be attributed largely to heredity from particular families rather than to the system of breeding.

Differences in Resistance among Inbred Families

Passing now to a comparison of the different inbred families with each other, we come to results which appear much more striking than the differences between inbreds and crossbreds. In all three groups of experiments, one family, 35, stands out as distinctly more resistant than the others. It leads the average of the others by 16 per cent. in lots 3–5, 19 per cent. in lots 7–9 and 43 per cent. in lots 10–14. The more striking result in the last case is probably due to the weaker inoculation. In spite of the large amount of variation in each case, the probable errors put the superiority of Family 35 beyond question. Among the other families, family 2 is on the whole the most resistant. Families 13 and 32 are about equally susceptible. Only one animal from family 39 was tested. This one was one of the first in its lot, no. 7, to die, indicating low resistance in this family also so far as conclusions can be based on such a slender basis.

Inheritance of Resistance among Crossbreds

If there are hereditary differences in resistance, one might expect to find differences among the crossbreds depending on the families which went into their ancestry. A preliminary test of this point was made as follows: The length of life of each crossbred (*CO*, *CA* or *AC*) was entered under each of the four grandparental inbred families. Thus an animal in experiment *CA*, whose sire

was a cross between families 35 and 2 and whose dam was of family 13, was entered once under each of the former families and twice under family 13. The averages of the entries under each family in this tabulation are given in Table XIII. It will be seen that in each group of experiments, family 35 has a distinct lead over

TABLE XIII

AVERAGE NUMBER OF DAYS LIVED BY THE CROSSBREDS DESCENDED FROM EACH INBRED FAMILY

Each crossbred is entered under the family of each grandparent.

Family	Lots 3, 4, 5		Lots 7, 8, 9		Lots 10–14	
	No.	Av.	No.	Av.	No.	Av.
35	30	27.9	38	30.3	63	64.4
2	65	25.9	56	26.8	116	51.4
13	45	24.8	49	26.0	72	48.7
32	33	24.4	21	23.9	51	49.0
39	28	26.0	16	24.9	39	48.0
Misc.	19	26.0	12	24.2	19	48.8

the others as ancestral to resistant crossbreds. Family 39 and the other families which entered into the ancestry of the crossbreds appear to rank with the more susceptible families 13 to 32. It will be seen that the rank of families 35, 2, 13 and 32 as ancestors of resistant crossbreds is the same as their own rank in resistance. These results are brought out more clearly in a tabulation in which all of the crossbreds are classified as half-blood, quarter-blood and zero-blood of family 35. The last class may be divided into half-blood, quarter-blood and zero-blood of family 2. The results in comparison with those for family 35, 2 and the others combined are given in table XIV.

The half-bloods of family 35 are distinctly superior to family 35 itself, and thus much superior to their other ancestral families.[4] In the three groups of experiments

[4] The preliminary study of another large series of animals (lots 15 to 21) shows that while the order among the crossbreds, as related to the presence of the blood of family 35 has been maintained, the advantage of the half-bloods over family 35 is absent, the curves being almost the same with the half-bloods slightly less. The order of the inbred families it may be added remains exactly as described.

TABLE XIV

THE AVERAGE NUMBER OF DAYS LIVED BY THE CROSSBREDS WHICH ARE ½ BLOOD, ¼ BLOOD AND HAVE NO BLOOD OF FAMILY 35, THE LAST CLASS BEING CLASSIFIED SIMILARLY WITH RESPECT TO FAMILY 2

The averages for Families 35, 2 and the other inbred families combined are given for comparison.

	Lots 3, 4, 5		Lots 7, 8, 9		Lots 10–14	
	No.	Av.	No.	Av.	No.	Av.
½ blood (35)	12	28.9	14	32.2	25	65.6
¼ blood (35)	6	28.7	10	24.8	13	56.5
No blood (35)	37	25.0	24	24.4	52	43.9
No (½ blood 2)	16	25.9	11	24.4	32	46.0
blood (¼ blood 2)	16	24.4	10	24.0	13	39.2
(35) (No blood 2)	5	23.8	3	25.7	7	43.6
Family (35)	9	25.7	9	27.1	22	58.5
Family (2)	28	23.1	13	22.8	27	45.3
Other Inbreds	18	20.4	26	22.6	23	35.6

3–5, 7–9 and 10–14, these half bloods exceed family 35 by 13 per cent., 19 per cent. and 12 per cent. respectively in duration of life, and exceed the other inbred families by 31 per cent., 42 per cent. and 60 per cent. respectively. We have here more decisive evidence of the improvement in vigor in this respect which may follow a cross, than in the comparison previously made between the total inbreds and total crossbreds. The most probable explanation is the same as that applied to the improvement in fertility, weight and death rate following crosses, viz., that each inbred family tends to supply dominant factors, favoring vigor, which are lacking in the other. Applied to the present case, this means not only that family 35 possesses dominant factors for resistance, lacking in the other families, but that some or all of the latter may possess such factors lacking in family 35. By inbreeding among the crossbreds, it should be possible to develop a strain even more resistant than family 35 provided that the linkage relations do not interfere with the fixation of the factors for resistance in one strain.

The quarter-bloods of family 35 confirm the conclusions derived from consideration of the half bloods. They are about intermediate between the half-bloods

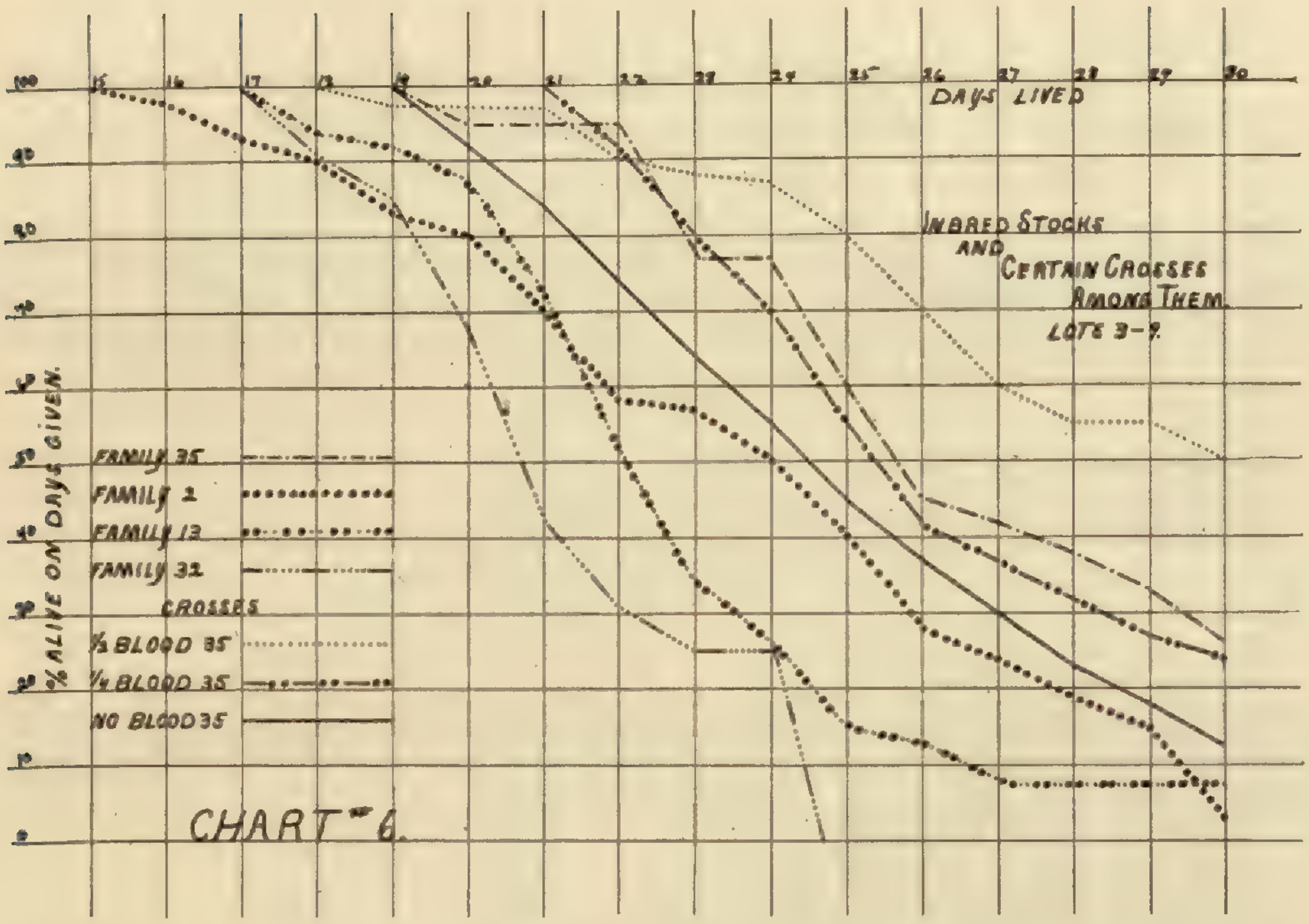

CHART 6. The mortality curves of the guinea-pigs of lots 3–9, grouped by breeding. Four inbred families. The crossbreds are grouped according to the amount of blood of the most resistant inbred family, No. 35.

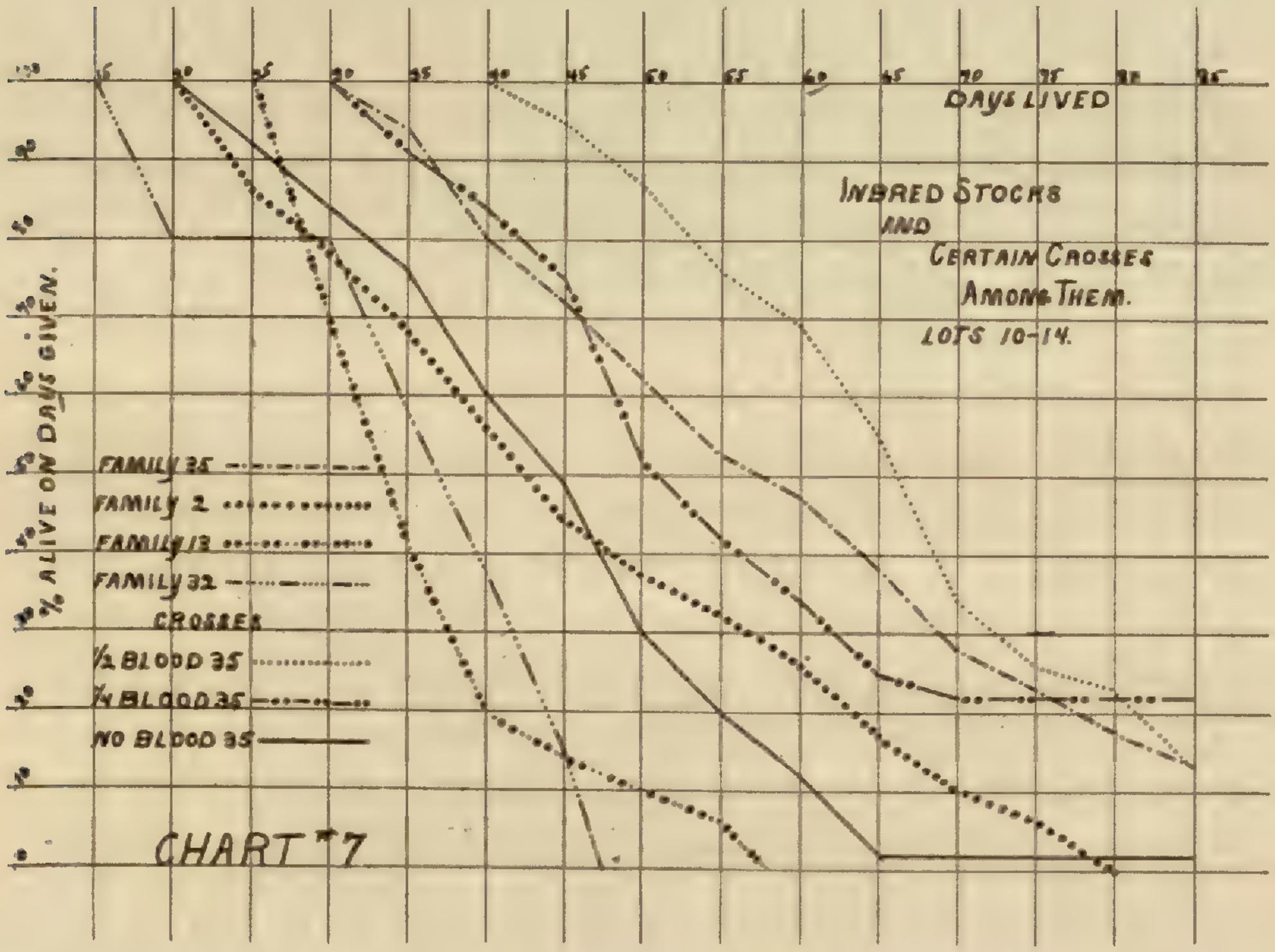

CHART 7. The mortality curves of the guinea-pigs of lots 10–14, grouped by breeding as in Chart 6.

and zero-bloods in lots 10–14 and in a combination of lots 3, 4, 5 with lots 7, 8, 9.

Curiously enough the crossbreds with no blood of family 35 do not show the improvement over their ancestral inbred families shown by the half and quarter bloods. They exceed the average of families 2, 13 and 32 by only 13 per cent. in lots 3–5, 8 per cent. in lots 7–9 and 7 per cent. in lots 10–14. They are not consistently superior to the best of these families, family 2. There seems to be dominance of resistance on the whole but no supplying of complementary factors for resistance such as was indicated in the crosses of family 35. This indicates that families 2, 13 and 32 are in the main susceptible because of the same genetic factors. The relations of the factors present in the different families is a question on which it is hoped more extensive evidence can be presented later (Charts 6 and 7).

Inheritance of Resistance and Sex

The crosses involving family 35 give some evidence on the inheritance of resistance from sire and dam. The average length of life in reciprocal crosses is given in Table XV.

TABLE XV

The Average Length of Life of Males and Females from Reciprocal Crosses Involving Family 35

	No.	Av. Days
♂ (35) × ♀ (misc.)	7♂	63.6
	5♀	59.4
	12	61.9
♂ (35 × misc.) × ♀ (misc.)	6	64.0
Total, (35) on sire's side	18	62.6
	No.	**Av. Days**
♂ (misc.) × ♀ (35)	7♂	73.6
	6♀	63.7
	13	69.0
♂ (misc.) × ♀ (35 × misc.)	7	50.1
Total, (35) on dam's side	20	62.4

The results are irregular, as might be expected with such small numbers, but they show that there is trans-

mission of resistance from each parent to offspring of each sex. The total figures—62.6 days where family 35 is on the sire's side, 62.4 where on the dam's side—indicate that there is probably equal inheritance from each sex.

DEGREE OF DETERMINATION OF RESISTANCE BY HEREDITY

We have shown that the length of life after inoculation was determined less than 2 per cent, by weight, age and rate of gain combined, in lots 7–9 and less than 7 per cent. by these factors in lots 10–14. It is interesting to compare the degree of determination by heredity with these figures. Such a comparison can be made for the crossbreds by finding the correlation between length of life and the amount of blood of family 35. The correlation in lots 3–5 comes out $+.319 \pm .082$, in lots 7–9, $+.539 \pm .069$ and in lots 10–14 $+.572 \pm .048$. For reasons which have been mentioned the latter two groups are much more satisfactory than the group of three miscellaneous lots, 3, 4 and 5. The average in lots 7–9 and 10–14 is $+.560 \pm .039$. The square of this coefficient, .314 indicates that over 30 per cent. of the variation in length of life is caused by heredity, neglecting such heredity as may be due to differences among families 2, 13, 32 and 39.

If we assume that some 10 per cent. of the variation is due to differences in condition, weight and age and over 30 per cent. to heredity, we still have over 50 per cent. of the variation due to unknown causes. From the nature of the case, however, a large amount of accidental variation is to be expected.

RELATIONS BETWEEN RESISTANCE AND OTHER CHARACTERISTICS

It is clear that family 35 is markedly more resistant to tuberculosis than the other inbred families, and that among these, family 2 is somewhat more resistant than families 13, 32 and probably 39. The question arises

whether these differences in resistance are related to any of the other characteristics in which these families differ.

We may dismiss color with a few words. Families 32 and 39 produce only animals of the primitive golden agouti color; families 2 and 13 produce only blacks, a color which differs from golden agouti by a single recessive factor; family 35 is composed of yellow agoutis. This color is recessive to golden agouti, depending on an allelomorph of albinism. We can not attribute the high resistance of family 35 to the yellow agouti color since the even more highly resistant crossbreds between family 35 and the other families are all of the golden agouti color also found in the susceptible families 32 and 39.

In all of the families there are varying amounts of white in a piebald pattern and of red or yellow in a tortoise-shell pattern. No influence can be attributed to the amount of white unless it is assumed that an intermediate condition is superior to either extreme. Family 39 has the least white, averaging less than 20 per cent.; families 13 and 32 have the most, averaging over 80 per cent.; while families 2 and 35 are intermediate with about 70 per cent. and 60 per cent. white, respectively. It may be added that the correlation between the amount of white in the coat and length of life in family 35, lots 10–14, was found to be virtually zero. Similarly, family 35 is intermediate between family 39 with least red and family 2 with most red in the colored parts of the coat.

The records in size, fertility and death rate among the inbred families and cross breeding experiments during the year 1919, in which all of these tested animals were born, is given in Table XVI. In this table, the weights and mortality records are corrected for the important effects of size of litter by calculating separately the averages in litters of 1, 2, 3 and 4 and finding an index in which these averages are weighted 1, 3, 3 and 1, respectively. This means practically that all of the records are reduced to the basis of an average size of litter of 2.50.

Because of the method of averaging, the figures are

not strictly comparable to those presented in Tables VII, VIII and IX for the animals actually tested. However, it is safe to conclude from a comparison of these tables that the animals tested were a fairly random selection from their respective families and experiments.

It may be noted in passing that the figures in Table

TABLE XVI

AVERAGES OF THE CHARACTERISTICS OF GUINEA PIGS BORN IN FIVE INBRED FAMILIES (2, 13, 32, 35 AND 39) AND CERTAIN CROSSBREEDING EXPERIMENTS IN 1919

CO is the first cross between inbred families. In *CA*, the sire is crossbred, dam inbred. *AC* is the reverse of *CA*. *B* is the control stock. The mortality and size characters are indices in which the averages for litters of 1, 2, 3 and 4 are weighted 1, 3, 3 and 1 respectively.

Family or Experiment	Number Young	Per Cent. Born Alive	Per Cent. Raised of Those Born Alive	Per Cent. Raised	Birth Weight (Grams)	Gain per Day to 33 Days (Grams)	Weight at 33 Days (Grams)	Size of Litter	Litters per Year	Young per Year	Young Raised per Year
2.....	331	80.3	88.9	71.4	76.9	3.40	189.1	2.43	3.82	9.30	6.46
13.....	259	87.1	85.2	74.2	88.4	4.76	245.4	2.85	3.79	10.79	6.67
32.....	146	90.3	78.8	71.2	82.2	4.13	218.6	2.25	3.48	7.81	5.30
35.....	258	80.2	84.7	67.9	88.3	4.57	239.1	2.37	3.68	8.72	5.64
39.....	72	93.7	80.7	75.6	77.9	3.79	203.1	2.40	2.97	7.13	5.15
Tot. Inbred.	1,066	84.0	85.1	71.5	82.8	4.12	218.9	2.47	3.63	8.97	5.96
CO.....	280	91.1	89.4	81.4	81.5	4.52	230.5	2.55	3.90	9.93	7.87
CA.....	302	85.8	94.5	81.1	84.3	4.53	233.8	2.72	4.24	11.53	9.20
AC.....	419	87.7	90.9	79.7	88.5	4.54	238.2	3.27	4.16	13.60	10.78
B.....	374	90.0	84.9	76.4	90.2	4.94	253.2	2.90	3.76	10.90	7.96

XVI give a good illustration of the statements made in regard to the effects of crossing, with the exception of a few records based on inadequate numbers.

From this table it will be seen that family 35, the most resistant to tuberculosis, held a rather low position among the inbred families in most other respects. It is actually the poorest in mortality among the young, and fourth in size of litter and first in nothing. There was thus no close relation between high resistance to tuberculosis and vigor in other respects in 1919.

However, the rank of a family in a single year is not always a safe indication of its true position genetically

in characters in which environmental conditions are of very much more importance than heredity. The rank of the families has been calculated for the periods 1906–1910, 1911–15 and 1916–19, making due corrections for the effects of size of litter. As previously stated, high correlations were found between the ranks in the first two periods in most respects among the 23 inbred families. Table XVII shows the ranks of the 5 surviv-

TABLE XVII

RANK OF FAMILIES IN RESISTANCE TO TUBERCULOSIS IN 1919, AND RANK IN OTHER CHARACTERISTICS DURING TWO PERIODS, 1911–15 AND 1916–19

Mortality and size characters are corrected for the influence of size of litter and (in 1916–19) for seasonal differences.

Family	Resistance to Tuberculosis	Per Cent. Born Alive	Per Cent. Raised of those Born Alive	Per Cent. Raised	Birth Weight	Gain to 33 Days	Weight at 33 Days	Adult Weight	Size of Litter	Litters per Year	Young per Year	Young Raised per Year
35....	1	3–3	3–1	3–1	2–2	2–2	2–2	2–2	1–2	2–2	1–1	1–1
2....	2	5–5	1–2	4–2	5–5	5–5	5–5	5–4	4–4	1–1	4–2	3–2
13....	3	2–4	2–3	2–4	1–1	1–1	1–1	1–1	2–1	3–4	2–3	2–3
32....	3	4–2	5–5	5–5	3–3	4–3	4–3	4–5	5–5	4–3	5–4	5–4
39....	3	1–1	4–4	1–3	4–4	3–4	3–4	3–3	3–3	5–5	3–5	4–5

ing families in the second and third period. The reality of the differences among these families is evident. Taking the following 6 characters—percentage born alive, percentage of these raised, birth weight, gain to 33 days, frequency and size of litter—the correlation between the ranks in the two periods is + .83.

The positions of the families agree in the main with those for the single year 1919. The position of family 35, however, is much better in most respects, a point which will be discussed later.

The difficulty of classifying the families in the order of general vigor is shown by this table. Families 2, 13 and 39 present curious combinations of high vigor in certain respects with weakness in others. It is true that there is perfect correlation between the ranks in size of litter and adult weight, but the order in which this places the

families, 13, 35, 39, 2 and 32, is far from being the order in frequency of litter or the mortality records. Taken as a whole, family 2 seems to have been rather the easiest family to keep going. Its regularity in producing litters and success in raising the young which are born alive are factors in this, but even more important seems to be another factor, probably correlated with that last named, the longevity of the animals after the matings are made, in which it far surpasses all of the others. Families 13 and 35 have also been relatively easy to maintain. This leaves families 32 and 39 as those most difficult to keep up to a desirable strength.

In spite of the better record of family 35 in the four years, 1916–19, as compared with the single year 1919, there is still no close relation between resistance to tuberculosis and vigor in other respects. In size, in both elements of fertility, and in the percentage of the young born alive, family 35 is still inferior to families which are distinctly more susceptible to tuberculosis. It may, however, be significant that family 35 led in the percentage raised of the young born alive and that family 2, second in resistance, is also second in this respect.

It may also be significant that while family 35 does not stand out in any particular element of vigor, unless in that last named, it stands relatively high in all, and so is the best family in the number of young produced per year by a mating, the product of frequency and size of litter, and is also the best family in the total percentage of the young raised, the product of the percentage born alive and the percentage of these raised. Moreover, family 2 is second in both respects as in resistance. These results suggest that while resistance to tuberculosis is not related to the most important factors which determine the various elements of vigor, it is a contributing factor to a sufficient extent to make the total efficiency of a resistant family higher than that of a susceptible family.

In this connection, the low standing of family 35 dur-

ing 1919 requires some consideration. The general condition of the stock was much better in 1919 than in any of the three preceding years. The principal cause of the high death rate, small and infrequent litters and slow growth during 1916, 1917 and 1918, especially during the first half of each year, was probably an insufficient supply of green feed during late winter and early spring. Symptoms, such as lameness, swollen and bleeding gums, were noted rather frequently. These were probably indicative of scurvy. A form of pneumonia was also rather common. Whether tuberculosis was present in the stock at this time is not certainly known. It is doubtful, as guinea pigs seldom take the disease unless directly inoculated. However, this may be, it seems probable that family 35 has a special ability to withstand exceptionally adverse conditions. It is not unlikely that this characteristic may be connected directly or indirectly with its resistance to tuberculosis. Under good conditions, on the other hand, there seems to be little if any relation between this form of vigor and apparent vigor in other respects.

Conclusions

There is little or no relation among guinea pigs between resistance to tuberculosis and sex. The present data indicate a possible superiority of the males, but one which is too slight to be certainly significant.

The data suggest a slightly greater susceptibility among the progeny of very young or very old females but this also is of doubtful significance.

Size of litter, birthweight and rate of gain up to weaning give virtually no indication of the probable length of life after inoculation.

The rate of gain preceding inoculation and the age and weight at that time all together determined less than 7 per cent. of the variation in length of life in a very heterogeneous lot of guinea pigs and less than 2 per cent. in a somewhat more homogeneous lot.

Marked differences in resistance were found among a number of inbred families of guinea pigs.

The high resistance of one of these families was transmitted by each sex to the offspring of each sex in crosses with other inbred families.

In crosses involving this most resistant family, the progeny were superior even to this family itself, indicating not only the dominance of resistance over susceptibility but possibly also the presence of complementary factors among the families.

In crosses among the more susceptible families, the progeny were little, if any, superior to the family of the better parent, indicating dominance but not complementary factors in this case.

Over 30 per cent. of the variation in length of life after inoculation among the crossbreds was determined by the amount of blood from the best inbred family. Allowing 10 per cent. of the variation as due to age, weight and condition, 50 to 60 per cent. remains as due to accidental and unknown causes.

The factors which determine the resistance of a family to tuberculosis are not closely related to the other elements of vigor, including rate of growth and adult weight, frequency and size of litter, the percentage of the young born alive and the percentage of these raised to weaning. There is some evidence that they are contributing factors to a sufficient extent to give the most resistant family a record above the average in each element of vigor and so give it the highest or nearly the highest total efficiency. Even this relation of resistance to other elements of vigor appears to be present only under exceptionally adverse conditions when it may play a direct part in determining the health of the stock.

The results in regard to resistance to tuberculosis are like those for other characteristics as regards the differentiation among families brought out by inbreeding, the improvement resulting from crosses between inbred families and the independence genetically from other elements of vigor.

GAMETIC AND OBSERVED RATIOS IN DROSOPHILA

DR. CALVIN B. BRIDGES

THE populations and families with which the geneticist deals are not the real objects of his investigation; for him, the distribution of characters is only an index of the preceding distribution of genes in gametes. But the whole course of embryonic development, with heavy mortality possible at every step, has intervened between the individuals that he classifies and the gametes from which they came. The observed classes correspond accurately to the original gametic series only in case this mortality is indiscriminate—that is, only if there is no differential viability.

In the breeding work with *Drosophila* there has been a continual effort to eliminate distortion in the ratios, which depends largely upon: (1) the extent of the mortality involved, this being characteristic in amount for each mutant type and character combination, (2) the suitability of the culture media and conditions, and (3) the competition when the number of developing individuals is in excess of the optimum number for the available food supply.

The problem of over-crowding (3) is simplest of solution, though over-crowding was the largest source of disturbance in most of the early work, as well as in some of the later. The remedy is, in the first place, to limit the number of eggs per culture to the output of a single female. No mass-cultures should be raised in experiments in which the ratios among the offspring are of importance. In the second place, as the larvæ grow larger and also increase in number with each day's output of eggs, the competition becomes intensified throughout the later stages of the culture. To meet this increasing demand, there must be fresh supplies of food, or enough food must be provided at the start so that even at the end there is sufficient for free development of all larvæ. In point of economy it is better to concentrate on a few cultures that are liberally supplied than to raise a greater

number that would mean optimum conditions for none and doubt concerning the reliability of all.

The main problem in connection with the environment (2) is to find a kind of food that will allow full development of even very weak classes. It is in this field that the greatest changes in method have been made. For some years—from 1910 to 1916—some modification of the fermented-banana method of preparing food was followed. Ripe sound bananas were peeled, and the pulp left for about 24 hours in a liquid containing yeast. This liquid was usually the fermented juice from the previous lot of bananas. About 25 grams of this fermented banana was put in the bottom of a culture bottle and covered with absorbent paper.

It was suspected that the real food of the larvæ was not primarily the banana but was rather the yeast cells and perhaps also the bacteria, the banana being mainly the culture medium for the yeast. This has been established by the work of Northrop,[1] of Loeb and Northrop,[2] and of Baumberger.[3] In July, 1916, in consultation with Northrop, I started experiments with a view to using as a culture medium standardized solutions, instead of banana. The solution was absorbed and held in a cake in the bottom of the culture bottle by shredded paper toweling, which offered extensive surface for the growth of the yeast. This method was unsuccessful; the flies laid few eggs and these were often overgrown by the yeast and killed. Esters and other chemicals with fruit odors did not lead to greater egg production. Perhaps better success with culture solutions would be obtained in supplementing and modifying banana methods.

The banana method was modified with a view to discouraging the growth of moulds and putrefactive bacteria by mild antiseptics or correctives, such as benzoate, thymol, formaldehyde, alcohol, powdered marble for neutralization of excessive acidity, etc. Good results were obtained with alcohol, where several extensive sets of comparative tests seemed to show that about 1.5 per cent. of alcohol in the food was desirable. The most successful alcohol method was roughly as follows: The pulp of sound ripe bananas was weighed and put with an equal number of c.c. of 3 per cent. alcohol in a shallow, covered dish. No yeast was added, since enough wild yeast was usually present. The food

[1] *Jour. Biol. Chem.*, 1917, pp. 181–187.
[2] *Jour. Biol. Chem.*, 1916, pp. 309–312.
[3] *Jour. Exp. Zool.*, 1919, pp. 1–28.

was at its best when it had fermented for about 24 hours. The optimum amount of drained banana was found to be about 25 grams per bottle. This was put upon the bottom of the culture bottle and one gram of paper-toweling strips (about 5. ×.7 cm.) was matted down on the top. Pint culture bottles gave a greater output per pair than halfpints. The alcohol method was used more successfully than the old method during the fall and winter of 1916.

In the spring of 1917 considerable work was done in testing out various media containing starch, sugar, peptone and salts. This method gave good results except that trouble with moulds was greatly increased.

In the autumn of 1916, Dr. R. W. Glaser told me of certain culture-media experiments that Mr. Baumberger and he were carrying out with banana infusions and agar.[4] Dr. Glaser later sent me directions for preparing these media and also some prepared tubes. My tests of the method showed that the amount of food was inadequate for general use, although sufficient for the small number of flies that they wished. I increased the concentration of the media by the addition of sugar, banana flour, etc., but principally by grinding up and using all the pulp of the bananas, instead of using simply the strained juice. A comparison of fresh banana with banana that had been fermented before incorporation showed that the fresh banana was superior. Likewise fresh banana was superior to banana raised to the boiling point at any stage of preparation. It was found that yeast should not be distributed throughout the media. Experiments showed that it is advisable to have a very light seeding of yeast confined to the surface of the solidified media. Also it is well to keep the yeast from the margin as much as possible, since fermentation at the sides and beneath the cake makes the cake break loose and rise. The amount of agar was found to be adequate at 1 per cent.

It was some months before this method was improved so far that it gave better results than those given by the old method or the alcohol method. In the spring of 1917 it was worked out well enough so that it was substituted for the old method in my regular work. By the winter of 1917 it had become quite

[4] *Science*, 1917, pp. 21–22.

generally adopted in the laboratory. Several points have been improved since, so that the procedure at present is as follows:

1. Use bananas that are thoroughly ripe or over-ripe.
2. Peel the bananas and weigh the pulp (100 grams of pulp provides for about four culture bottles).
3. Weigh agar-agar, 2 per cent. of amount of banana.
4. Measure as many c.c. of water as there are grams of banana.
5. Add agar-agar to water and heat until the agar has dissolved. (Complete solution is hastened by the addition of a small amount of fresh water soon after the boiling point has been reached.)
6. While the agar is heating, press the banana through a potato masher or a coarse sieve, and place in readiness the bottles (which should have been previously washed and also preferably steam sterilized). Get ready yeast (Magic Yeast ground up) and paper (absorbent paper, paper toweling cut into 4-fold squares $3'' \times 2''$) and cotton (stoppers may be reused, but should be dry sterilized by enclosing over formalin. Cotton stoppers are better if made rather tight and covered with very soft cheese-cloth).
7. Stir banana into hot agar solution. Mix thoroughly. Mixture should not be heated any longer.
8. With ladle and funnel pour about 50 c.c. of the media into each half-pint or pint milk bottle. (The media should be at least $\frac{3}{4}$ in. thick to stick well.)
9. Sprinkle top lightly with dry yeast.
10. Put in contact with media a 4-fold square of absorbent paper.
11. Stopper with cotton.
12. Use same day. Best to use as soon as cool. Not good after two days.

Flies can be mated in vials and then transferred to the culture bottles at the end of the day. A little food may be kept going by the alcohol method for use in vials, for covering over mould patches in culture bottles, and for refeeding stock cultures.

The distortions in the ratios that arise from mortality characteristic of given mutants and combinations (1) can not be eliminated by direct methods. Fortunately, a large proportion

of the mutants are little if at all below normal in degree of viability; that is, when such mutants are compared with the wild type under identical conditions, the observed ratios show little or no deviations from expectation beyond those due to random sampling. As an example may be mentioned white-ocelli, which is known to have maintained itself with practically undiminished frequency through 175 generations of competition, under unfavorable conditions of culture,[5] with the wild-type. In the main, the mutant races that show normal viability are those whose somatic effects are "slight". Thus, white-ocelli affects the color of the tiny group of three ocelli on the top of the head. The character, though involving so small an area, is perfectly sharp and definite, and under proper conditions of illumination and magnification is fairly easy to classify. The same is true of many other "slight" mutations, such, for example, as speck, cross-veinless, and hairy, which are among the most valuable *Drosophila* mutations. On the other hand, mutants that involve more extensive or manifold changes, such as club, notch, rudimentary, and delta, are also among those poorest in viability. Some of these changes in themselves interfere with the success of the individuals possessing them: flies with "spread" wings or "dachs" legs are liable to become caught in the culture media and die. These changes are also sometimes obviously accompanied by serious internal derangements. In the case of streak, for example, it can be seen that the internal muscles of the thorax are largely replaced by bubbles and blood sinuses. The correlation between inviability and the extent of the visible change is high, but is lessened by the cases in which the internal accompanying changes are of slight disadvantage. Thus, the mutant "pads" resembles "club" very much, and appears to be a greater change in the same direction, but is nevertheless far freer from inviability. Conversely, certain mutants that are usually lethal occasionally do produce offspring, which are then not as strikingly different from the wild-type as some other mutants that have good viability. Lethal-10 very occasionally survives, and the individuals are scarcely to be distinguished from dwarfs of a certain mutant race (dwarfoid) that is little inferior to the wild-type in viability.

[5] *Biol. Bull.*, 1920, pp. 231–236.

The connection between observed character-change and inviability is even more indirect than suggested above. In the *Drosophila* work it is not the comparative viability of adults possessing given character differences that is of the most importance. Even though many of the characters are of such a nature that their possessors would be under a serious handicap in competition, in relatively few cases does this fact lead to alterations in the observed ratios, since the classifications are made usually soon after the flies hatch, *i.e.*, every 24 to 48 hours. It is true that certain mutant forms such as "divergent" and "gull" and "bifid" wings, also "dachs" and "reduplicated" legs tend to become entangled in the culture media and drowned immediately after emergence, so that in these cases the observed ratios are somewhat different from the hatching ratios. There are also a few mutants—mostly semi-lethals—in which the adult is unable to live very long even under the most favorable conditions. Among these may be mentioned "lemon," "apterous," and especially "decrepit." The "decrepit" flies die a few hours after hatching in spite of all care in helping them emerge from the pupa case, in keeping them in quarters not too dry or wet, and in suplying them with suitable food. It would seem that the death of such flies as are obviously weak on hatching is to be referred to difficulties encountered in the pupa stage.

Even inviability arising in the pupal stage, like that in the adult stage, is less general and significant than that in the larval stage. Most of the inviability that affects the ratios of adults is to be referred to differences acting in the larval stage, as is evident from comparative studies of the results of pair and mass cultures and of changes in culture methods that affect only the larval period. The difference between mass and pair cultures is essentially a difference in the number of larvæ that are in competition, the food conditions and the character of the larvæ being at first identical in the compared cultures. It is found that the distortion to the ratios among the adults is roughly proportional to the number of larvæ in competition. How extreme such competition may be is evident from the fact that a point is soon reached after which further increase in the number of mothers brings no increase in the number of progeny and may even result in a decrease. So predominant is the larval stage in its influence upon viability that the chief field of

improvement of culture conditions has been that of the character and methods of use of the food for the larvæ. There are specific viability differences among the larvæ of the different mutant types and combinations. Such viability differences must depend upon differences in the characters of the larvæ, and these, because of the intervening metamorphosis, have little direct relation to the characters of the adult, but are products of the action of the same mutant gene. The high correlation observed between extensive change in adult characters and high degree of inviability must, then, mean that such genes generally cause changes which interfere directly with the success of the larvæ.

Three larval characters are known—the tumor responsible for the death of lethal-7 larvæ, the much shortened larvæ of the mutation "chubby," and a marking on the posterior end, viz., "barette." It is supposed that a high proportion of the larval characters that lead to inviability are differences in internal structures, but some of these might be detected. However, no systematic search for larval characters has been made even in the case of the inviable mutants where such differences are probably present.

As we have stated, the distortion in ratios that arises from inviability, especially inviability originating in the larval stage, can be very materially reduced by improvements in culture media and in methods. Many poorly viable mutants can be made quite generally usable, as, for example, dachs. But when a mutant such as dachs is to be used in linkage determinations, the experiments should be so planned as not to include more than one of these characters. The presence of a single poorly viable character in an experiment does not prevent the calculation of correct crossover values. The complementary classes that do not include the inviable character should be in the same proportions as in the gametic series. Even in cases of mutants completely lethal, the linkage relations of the lethal gene can be calculated accurately from the ratios shown by the other characters of the cross. The classes that include the inviable character are often also usable, but with less certainty that the values are correct. Such values are correct when the presence of the mutant decreases by the same percentage the size of every class in which it occurs. The fewer the mutants involved in an experiment, the greater the likelihood that this

result will follow. Unexpected irregularities in ratios may arise where many mutant characters are distributed in different combinations. These peculiarities of inviability are probably comparable to "specific" and "disproportionate" modifications in eye-color, etc.[6]

If more than one poorly viable mutant is present in a linkage experiment, there is distortion in the ratios due to linkage, and such experiments are either entirely worthless, or are only to be regarded as rough indicators of the real relations. As we saw, if only one inviable mutant is present in a cross, one of each of the pairs of complementary classes remains undisturbed; and correct values can be calculated from them. The presence of a second inviable mutant leaves undisturbed only that class in which neither mutant occurs. The calculation of crossover values under this circumstance is somewhat comparable to solving for two unknowns with a single equation. Solutions can be obtained only by assuming some relationship between the two disturbances. Thus, we may assume that the disturbances are independent; that is, that there is no specific interaction of the kind mentioned above, and the class in which both mutants occur is accordingly of the size that would be expected from the amount of the disturbance present in those classes in which each occurs by itself. On this basis, the crossover values are calculated from the square root of the product of the two complementary non-crossovers, and likewise of the crossover classes, instead of from the sums of such complementary classes.[7] The assumption of independence would be approximately correct in perhaps a majority of crosses in which only two or a few loci are involved. If the disturbances are related—if they tend to neutralize or to exaggerate each other—a correction can still be made by raising an equal number of individuals in the complementary cross. In the two complementary back-crosses, a × b (repulsion) and a b × wild-type (coupling), the character combinations that are non-crossover classes in the repulsion experiment are crossovers in the coupling experiment, and vice versa. If the presence of a particular class has given a crossover value too high in the one cross, then it will give a value correspond-

[6] *Jour. Exp. Zool.*, 1919, p. 374.

[7] This geometric mean method was proposed by Muller, who gave an excellent discussion of the difficulties involved in differential viability. (AM. NAT., Vol. L, p. 351 ff.)

ingly too low in the complementary cross, and the mean value will be correct.[8]

Since the effects of inviability are likely to be more pronounced, even disproportionately so, as the number of mutant characters present simultaneously is increased, it is advisable to plan any linkage experiment in which several characters are to be involved, in such a way that the characters are distributed as evenly as possible. The type of back-cross that gives the evenest possible distribution, as well as the smallest proportion of individuals in which the higher combinations occur, is that in which half the mutants have entered the cross from one parent and the other half from the other parent, and in which the mutants are *"alternated"* as regards their positions along the chromosome. Thus, for example, let us consider a back-cross in the third chromosome in which the seven mutants to be used are: roughoid at 0.0; hairy at 25.8; scarlet at 35.1; dichæte at 38.5; pink at 44.6; spineless at 54.2; and ebony-2 at 66.9. The two parents should be roughoid scarlet pink ebony-2, and hairly dichæte spineless; and the formula for the F_1 multiple heterozygote would be:

$$\frac{\text{ru} \qquad \text{st} \qquad \text{p} \qquad e^2}{\text{h} \qquad \text{D} \qquad \text{ss}}.$$

The production of an individual possessing all seven characters would require an hexuple crossover, which almost certainly would not occur.[9]

A method that overcomes inviability effects to the greatest extent where many mutants are involved, but which unfortunately requires too great labor for general use, was devised by Muller.[10] F_1 females heterozygous for any number of mutants are crossed, not to the multiple recessive as in the ordinary back-cross, but to the wild-type. Except for the dominant mutants, none of the characters involved appears in the resulting individuals, and hence do not exert injurious effects. These indi-

[8] This "balancing of the inviability" has been discussed at greater length by Bridges, *Jour. Exper. Zool.*, 1915, p. 3 ff.; *Jour. Exper. Zool.*, 1920, pp. 281, 288; by Morgan and Bridges, Carnegie Pub. No. 237, p. 19, 43; and by Muller, AM. NAT., 1916, p. 353.

[9] See Bridges, *Jour. Exper. Zool.*, 1920, p. 295, for discussion and other examples of the "alternated back-cross."

[10] AM. NAT., 1916, p. 354 ff.

viduals have then to be tested singly to determine what recessive characters they carry and hence to what crossover category they belong.

Thus, by improvements (1) in the type of experiment planned, (2) in the culture media and methods used, and (3) in the method of calculation, disturbances in the ratios can usually be held within negligible amounts.

To these indirect methods of obtaining accurate values is to be added one still more important—namely, the discovery of new mutants in which viability is practically normal, and which can be substituted for mutants less satisfactory in that regard. Many of the loci are represented by several mutant allelomorphs, which often are different in viability as in other characteristics. Thus, of the eight cut allelomorphs, or appearances, cut-6 is distinctly the most nearly normal in viability. Likewise, of the five or six allelomorphs of the truncate locus, "dumpty" is the most satisfactory.

In most of the more complex linkage problems, especially those involving linkage-variations or coincidence, the behavior of particular regions of a chromosome is being examined, and the particular loci utilized are only indices of the behavior. What is most essential, therefore, is that there be workable mutant loci distributed rather evenly over all regions of the chromosomes. As the number of mutants in a particular region increases, there is a greater range of choice and greater probability that one or more of the mutants of that region will have normal viability. Thus, bifid (at 7.3) was long the only workable mutant at a favorable distance from the left end of the X-chromosomes. More recently ruby (at 7.5) because of its better viability has displaced bifid from general use, and this in spite of the fact that ruby interferes with the classification of several other eye-colors (especially prune and garnet) while bifid is workable with nearly all other mutants.

In regions less well represented in numbers of mutant loci, a mutant with excellent characteristics may be used rather than one whose position is more favorable but whose other characteristics are poorer. Thus, "humpty" is very favorably located in the second chromosome in the middle of the long region from curved (73.5) to plexus (98.5), but its viability is so poor that in most experiments it is better to leave this section unfollowed than to introduce humpty. There are at present few regions

that are not satisfactorily represented. By far the longest of these in the 25.8 unit interval from roughoid to sepia in the left end-region of the third chromosome. Because of uncertainties in classification, it is not ordinarily possible to use more than two eye-color mutants together in an experiment, and such masking of one character by others affecting the same organ leads to a continued search for combinations of characters that can be handled simultaneously. In general, the slight mutant characters mask each other less than do extreme ones, and these are, usually the least inviable. There is a continual improvement of the working material by the substitution of better mutants.

A PROBABLE EXPLANATION OF POLYEMBRYONY IN THE ARMADILLO

PROFESSOR CHARLES R. STOCKARD

CORNELL UNIVERSITY MEDICAL COLLEGE, NEW YORK CITY

BY arresting the development of the fish's egg during early stages double individuals and twins are frequently induced. The interruption or arrest makes it possible for more than one potential growth point along the germ-ring to give rise to an embryonic shield. In other words, accessory invaginations or blastopore formations occur as the initial structural step in doubleness. The interruption in the development of the fish embryo must be introduced during the cleavage stages and before gastrulation in order to produce such phenomena. Among hundreds of eggs arrested during later developmental stages no double monsters or twins ever occurred. A complete account of these experiments is soon to be published but for our present purpose two facts are important: First, accessory embryo formations result from arrests in the developmental process; and second, the arrest must occur before gastrulation has taken place.

In the light of these experiments it has seemed possible to interpret somewhat more clearly than has formerly been done the remarkable phenomenon of multiple embryo formation in the armadillo.

On examining the uterus in two pregnant specimens of a South American armadillo von Jhering, in 1885, discovered that each contained eight fetuses enclosed within a single chorion. He correctly concluded that all of the fetuses in each mother had been derived from a single egg by some process of division into separate embryonic rudiments. After this valuable discovery and interpretation, the study of the armadillo's development lapsed and nothing of importance was added for almost twenty-five years. Two series of investigations were then begun simultaneously one on the Texas armadillo by Newman and Patterson,[1] and the other on the South American species by Fer-

[1] H. H. Newman and J. T. Patterson, *Jour. Morph.*, Vol. 21, p. 359, 1910.

nandez.[2] The growth and expansion of these twin studies has brought our understanding of the phenomena of polyembryony in the armadillo to a considerable state of maturity.

These authors readily agreed that in most species of armadillo the individual members of a litter, usually four in the Texas species and eight in the common South American form, are all derived from a single egg. It required considerable effort, however, to obtain the material that would furnish the morphological stages of the process by which the polyembryonic development was accomplished. We are finally indebted to Patterson,[3] for the very thorough and satisfactory manner in which he has collected and studied the early embryonic conditions; and particularly for having shown the first stages of the budding process through which the single blastocyst gives rise to four distinct embryonic areas, each exhibiting a typical primitive streak region.

In connection with the fish experiment it now becomes important to ascertain exactly what degree of development has been attained by the armadillo blastocyst at the time the budding process begins. And since, according to my interpretation, these buds should arise at the time of gastrulation or blastopore formation, it becomes necessary to consider very briefly the germ-layers and gastrulation in mammals. The decidedly precocious and highly modified method of forming the primary germ-layers in the mammalian blastocyst is not strictly comparable to gastrulation or the method of germ-layer formation found among the other vertebrates. On the other hand, the embryonic line or primitive streak of the mammalian egg is exactly comparable to the blastopore and head process formation in the simpler forms.

The blastocyst of the armadillo has already, by a process of cell migration and delamination, separated off the primary entoderm from the ectoderm and further modified these layers before the budding which forms the embryonic primordia has begun. The primordia are first formed by a thickening of the ectodermal layer of the blastocyst. The primary entoderm then invaginates into the primordia to form the secondary entoderm of the gut. The precocious cell migration and splitting into layers in the mammal's egg is associated with the early implanta-

[2] M. Fernandez, *Morph. Jahrb.*, Bd. 39, p. 302, 1909.

[3] J. T. Patterson, *Jour. Morph.*, Vol. 24, p. 559, 1913.

tion of the embryo upon the uterine-wall of the mother, and the later primitive streak formation may be interpreted as related to the actual gastrulation or blastopore formation away from which the line of the embryo always develops.

Whether the validity of the above briefly outlined interpretation of the germ-layer formation is admitted or not, we have in the armadillo a process of budding taking place from the blastoderm and associated with accessory or extra blastopore formation in much the same way as are the accessory embryos along the germ-ring in the egg of the bony-fish. These buds also accord with Kopsch's description of a double gastrular condition with two blastopores in a blastoderm of Lacerta agilis, from which he concluded that twin formation as well as anterior duplication arises from a double Einstülpungen. And further, Assheton has described a similar condition in a blastodermic vesicle of the sheep. He, however, imagined the condition to have been due to a splitting during the morula stage.

The double primitive streaks in the hen's egg and other forms all lend themselves to strengthen the interpretation that double embryo formation first asserts itself by a double gastrulation or blastopore formation, which is initially a process of double instead of single bud formation. Patterson's description of the origin of the quadruplet buds in the Texas armadillo furnishes the most striking case in the study of these conditions. And we may conclude that the budding or accessory embryo formation in the egg of the armadillo is exactly the same developmental process as that which gives rise to twins and double individuals in other vertebrate eggs.

However, the very important question yet remains to be answered. Why does this accessory bud formation occur so constantly in the Texas armadillo in contrast to the single embryo formation of mammalian eggs in general? Patterson failed to answer this question, but he supplied some very significant data which Newman,[4] has appreciated as being intimately connected with the occurrence of polyembryony.

In connection with the collection of material Patterson[3] discovered a "period of quiescence" of the embryonic blastocyst. Regarding this he states:

> The fact was first made apparent in 1911, when, after I had started collecting two weeks earlier than in the preceding year, I failed to

[4] H. H. Newman, "The Biology of Twins," Univ. of Chicago Press, 1917.

obtain the cleavage stages, although judging from the condition of development in the vesicles collected in previous years, one would naturally expect to find these early stages during the period of my first collection in 1911.

The following year he began collecting still two weeks earlier and again had a similar experience.

Practically all of these vesicles lie free within the uterine cavity, either in the horizontal groove or in the region of the attachment zone (placental area).

It is evident from these data that the embryonic vesicle remains for some time lying free within the uterine cavity. Just how long this period lasts, I am unable to state; for practically every old female taken at the earliest date (October 15) at which I have collected, possesses a free blastocyst. . . . Taking all the facts into consideration, I estimate the "period of quiescence" to last about three weeks; that is, from about the middle of October to the third or fourth of November.

In a study of sections no mitotic divisions were found to occur in the blastocysts during the "quiescent period."

The only point of interest cited by Patterson in connection with this peculiar phenomenon of interruption in development, was the fact that in no other mammal, except the deer, had such a condition been found. Bischoff had long ago, 1854, reported a "period of quiescence" lasting for some weeks during a so-called morula stage of the deer embryo.

Newman[4] has recognized the importance of Patterson's discovery of a "quiescent period" during the early development of the armadillo, and states in a discussion of twin formation that this "period of quiescence" probably, "holds the clue to the physiological explanation of polyembryony." In this position Newman is, in my opinion, largely right, but this is as far as the data led him, and he finally remarks:

> The problem is to locate the factors responsible for the slowing down of the developmental rhythm. Whatever these factors may be, and we have no definite knowledge of them, the result of retardation is polyembryony.

Newman thus fails to appreciate the second point in Patterson's discovery, and that is that the blastocysts always lie free in the uterus during the "period of quiescence." This fact enables us to go one step further since the lack of attachment and, therefore, lack of oxygen supply are very probably "the factors responsible for the slowing down of the developmental rhythm."

The armadillo egg like that of most mammals undergoes its early development in the fallopian tube and is, therefore, capable of reaching the blastocyst stage on its initial oxygen supply. After this time, however, it must become attached to the uterine wall for a further source of oxygen. For some reason in the armadillo the reaction between the blastocyst and the uterine wall is postponed, and the blastocyst is incapable of further developmental progress until this reaction is established and the necessary supply of oxygen becomes available. In exactly the same way the development of the blastoderm in the fish's egg is experimentally retarded or stopped by reducing the available oxygen supply and is again made to resume its development by supplying oxygen. In the case of the fish egg, the supply of ordinary nutriment is certainly not involved, and reactions similar to those of the armadillo egg are only obtained as responses to changes in temperature and rate of oxidation.

In the armadillo egg I also do not believe the retardation is of the nature of a starvation phenomenon, since we see nothing of the kind in other forms. Temperature changes are ruled out, since the temperature of the uterus is more or less constant. The absence of oxygen necessary for the energetic process of cell division, is, therefore, in all probability the arresting cause, and the retardation results in polyembryony.

Thus Patterson has found the developmental interruption to exist, and he has also shown the blastocyst to be disconnected from the uterine wall and its necessary oxygen supply during this time. However, he has furnished no data bearing on the reason for the delay in uterine reaction and the consequent failure of immediate implantation of the blastocyst such as normally occurs in other mammals. However, from what is known of the dependence of uterine reactions on conditions in the ovary (Leo Loeb,[5] Stockard and Papanicolaou[6] and others) it may very probably be that some peculiarity in corpora lutea formation is primarily responsible for the entire series of reactions leading to polyembryony in the armadillo.

The consideration of the armadillo egg up to this point has taken account only of the external factors influencing its mode of development. It must now be remembered as a fact of serious

[5] Leo Loeb, *Jour. Morph.*, Vol. 22, 1911.

[6] C. R. Stockard and G. N. Papanicolaou, *Am. Jour. of Anat.*, Vol. 22, 1917.

importance that the production of quadruplets from the single egg of the Texas armadillo is an almost constant occurrence, while the experimental attempts to produce twins and double individuals in fish eggs and other forms have given at best only small percentages of such individuals among the large groups of eggs treated. It is also a fact that all eggs do not furnish equally favorable material for artificial twin production. The eggs of the trout seem unquestionably more disposed to give rise to twin formations than do the eggs of Fundulus. Thus some eggs would seem to have a hereditary or truly innate predisposition towards polyembryonic formations. There is much reason to believe that aside from the external factors discussed, the armadillo egg itself is highly disposed toward the formation of accessory embryonic buds.

There is the possibility, of course, that this natural experiment with the armadillo egg has become so exactly regulated as to influence the developmental processes precisely the same way each time, yet this is highly improbable. The armadillo egg is not a case of simple twin growths from the blastoderm, but as Patterson finds, there are primarily two buds, and then very promptly two secondary ones arise making the four and after this the budding process ceases. In the South American species, however, it would appear as though a tertiary budding occurred giving the usual eight embryos; and in rare cases still another budding occurs from a few of the existing buds giving a total of as many as twelve. It would certainly seem as though the blastoderm in these species passes through a stage of agametic reproduction or budding of a nature unknown among other higher vertebrates. But the possibility for such expression might only exist on account of the delay in implantation of the blastocyst and consequent shortage of the oxygen supply necessary for the rapid formation and growth of the single embryo.

It is important to keep in mind that there are species of the armadillo which produce only a single offspring from one egg. It is not known whether their embryos have a "period of quiescence" but if they have, the period either occurs at a different developmental stage or the eggs do not possess the inherent budding tendency of the other species.

We have further to acknowledge the fact that although the egg of the deer has a "period of quiescence" during its development it does not give rise with any degree of frequency to twin indi-

viduals. In the first place it is entirely uncertain from the scanty accounts as to what time in development the quiescent period occurs. Assuming that such a period does exist, it might occur at some indifferent stage when no peculiar result would be expected, for example after gastrulation, as it does in the bird with no subsequent effect. In the light of the experimental production of double individuals it is readily understood that even though the egg of the deer is interrupted in its development at an early stage, it might still be capable, on resuming development, of giving a normal single embryo. A study of the experimental production of twin and double individuals among fish leads one to be surprised at the case of the armadillo, and to expect the reaction found in the deer. The constant interruption occurring in the development of the birds and other animals at indifferent developmental moments with no subsequent ill effects, renders commonplace the fact that the deer successfully withstands an interruption during its development without noticeable modifications in structural response. A full consideration of the different results following interruptions at critical and indifferent developmental moments will be published in a forthcoming number of the *American Journal of Anatomy*.

In conclusion we may summarize the cases as follows: The development of the armadillo is interrupted on account of a failure to become promptly implanted on the uterus and a consequent exhaustion of the available oxygen supply. The interruption occurs at a critical period just preceding the primitive streak and embryonic line formation. This egg appears to have a decided tendency under conditions of arrest to form accessory embryonic buds. As a result of the interaction of these external and internal forces polyembryony is produced.

In the case of the deer only one probable fact is known, and that is that a "period of quiescence" occurs. It is uncertain at what stage the arrest takes place but it is probably due as in the armadillo to a delayed implantation of the blastocyst. Either on account of the stage of arrest, or a lack of tendency to form accessory embryo-buds a typically single individual arises from this egg. The external factors may be the same as in the case of the armadillo, but they interact with different internal factors or different developmental moments to give a very different result.

NOTES AND LITERATURE

North American Early Tertiary Bryozoa. By Ferdinand Canu and Ray S. Bassler. Smithsonian Institution. United States National Museum. Bulletin 106. 1920, 879 pages, 279 text figures and 162 plates.

Students of both fossil and recent bryozoa will greet with interest and pleasure this monumental work long anticipated and recently issued, for while treating primarily of fossil bryozoa this monograph contains much of interest to students of living forms. This work appears in two volumes, one containing the text and text figures, the other consisting of photographic plates alone. A cursory inspection reveals the fact that these volumes possess the excellence of copious illustration, a most satisfactory virtue in the eye of those who will use them. The text figures are abundant, the number as stated above (279) by no means giving a true idea of the actual number, since each figure consists of from two to ten or more illustrations, representing portions or organs of the species under discussion, and often besides figures of nearly related species for comparison. From this point of view the number given should be multiplied many times, and by actual count the first fifteen text figures contain more than one hundred separate drawings or preparations. These are all either original with the authors or are taken from the illustrations of other bryozoologists. Each photographic plate likewise contains from twelve to twenty-five separate photographs. These are distinguished by a remarkable clearness and definiteness of outline, even of minute details, revealing an unusually skilful management of light and shade and producing an excellent and expert piece of work which will not fail to call forth the gratitude as well as the admiration of their fellow workers.

Over 700 species belonging chiefly to the two orders, Cyclostomata and Cheilostomata, are treated in this monograph. In the seventy or more pages of introduction the authors present many topics of interest involving new points of view which will doubtless stimulate further research. Of these topics but three will be touched upon.

1. It is gratifying to find clear definition and illustration of

the terms of the more recent nomenclature used in description and classification. The study of the bryozoa has developed to such a degree in recent years and so many new terms have been introduced which are found only in the scattered writings of numerous authors, that a new compilation and exposition similar to the classic work of Hincks in his "British Marine Polyzoa" would be useful. As far as it was compatible with the limits of this treatise such a compilation and exposition have been accomplished here, and both the beginner in the study of the bryozoa and the advanced worker will find great assistance in the discriminating use of the newer anatomical terms.

2. Of the general functions of the bryozoa, of the Cheilostomata especially, the discussion of the hydrostatic function is perhaps the most interesting because it presents the newest and latest views on this puzzling subject. The extrusion and retraction of the polypide, the action of the operculum and of the zoœcial muscles in these activities, and the relation of these to the ingress and egress of water was long a puzzle. Jullien in 1888 first discovered the so-called compensating sack or compensatrix under the dorsal surface of the zoœcium. Since then scattered studies have been made on this organ which was soon found to be present in many species. The present authors have continued this study and following Levinsen (1909) have made the presence or absence of a compensatrix the basis of division of the Cheilostomata into two sub-orders Anasca, without such a compensating sack; Ascophora, possessing such a sack.

In addition to the zoœcial hydrostatic system discovered by Jullien, the senior author, in 1915 discovered a zoarial hydrostatic system in the Anasca. This investigator found that the space under the ectocyst, in certain species lacking a compensatrix, was continuous from zoœcium to zoœcium throughout the colony. Into this space water is introduced or expelled thus compensating for the egress and ingress of the polypides. By such means minute creeping zoaria as the Lunulites, *e.g.*, are enabled to maintain themselves on the algæ on which live.

3. In line with their insistance on the value of function it is not surprising that these authors classify the Tertiary bryozoa on a physiologic rather than a morphologic basis as is the method followed by the older investigators. Believing as they do that (p. 70) "In the bryozoa, as in other living beings, the form is only the result of functions; therefore in the study of

morphological variations of the organs, we now substitute that of the physiologic functions. Our studies are therefore always directed toward the discovery of functions which modify the skeletal form." With this frank statement of the primacy of the Lamarckian principle, family, genus and species are thus briefly described:

The family is characterized by the larval form.

One genus differs from another in possessing a different function. The three essential functions of all bryozoa are:

1. Passage of egg and escape of larvæ (= rapport of operculum and ovicell).
2. Hydrostatic system and extrusion of the polypide (= form of the aperture and rapport of operculum with compensatrix).
3. Calcification and chitinization (= nature of the skeletal part and of the frontal considered as deposits of the endocyst).

Specific characters include all morphological variations and all of the characters of adaption.

Whatever philosophic views one may hold in regard to the relation between form and function, it is apparent that the characters chosen for family, genus and species present a uniform, logical system and constitute a good workable plan or hypothesis which it seems more than worth while for all workers in this field to attempt to apply. It must be remembered, however, that while in the class bryozoa, larval characters may afford valid data indicating relationship such characters have failed to afford satisfactory data of relationship among some other class of animals. Granting the validity of the assumption, however, the research necessary to establish this statement can be conducted only on living species. This, then, is a matter of immediate and pressing interest. The older bryozoologists considered the operculum a family character. In the present system the operculum becomes a generic character, changes in it being induced by changes in the essential zoœcial functions 1 and 2, that is, depending in part upon the relation existing between the operculum and ovicell, and in part upon the relation existing between operculum and compensatrix together with the form of the aperture. Here again, although much investigation has been conducted to verify these generic principles among the fossil Tertiary species, work on living forms should be undertaken to

discover how far recent species actually conform to the plan proposed.

Identification and description of the species of this collection constitute the main portion of this monograph. A superficial examination of the text reveals the fact that in order to apply these principles to present day bryozoa, momentous and wide spread changes will be necessary. Not only will genera and families be broken up, but many heretofore considered widely separate will be regarded as closely related and vice versa. While one is startled by the number and significance of the changes involved, yet the present morphologic method is so unsatisfactory that this attempt to apply a unitary principle which promises so to simplify classification and to lift it out of chaos, should be heartily welcome. Too high praise cannot be accorded the authors of this monograph for the excellence of this work so full of new and stimulating ideas.

ALICE ROBERTSON

SEATTLE, WASHINGTON

SHORTER ARTICLES AND DISCUSSION

THE EFFECT OF YEAST ON THE UTILIZATION OF FOOD BY WHITE MICE.[1]

In the present paper the question of the effect of the so-called vitamines on basal metabolism is considered and a procedure is indicated whereby it is believed more conclusive data may be obtained on the question. Preliminary experiments are described illustrating the method.

Several years ago Hopkins[2] in a carefully carried out experiment investigated the effect of a small addendum of milk to a diet of purified food stuffs. In brief his method was to feed in pairs two sets of young rats of the same origin, weight, etc., on a basal vitamine-free food and to one set give a small addendum of milk and determine the food intake and growth increment. By comparing the energy consumption and growth increments of the two sets of animals and by comparing these factors on the same set of animals after reversing the diets he was able to show "that the small milk addendum reduced the food consumption for a given weight increment to one half or less." In other words the vitamine increased greatly the animal's power to utilize its food in the production of growth. And as he showed that this was not due to difference in absorption from the intestines, the vitamine must have an effect on some factor involved in basal metabolism.

A number of the criticisms that could be made of Hopkins' method have been discussed frankly by him and there is left little or no doubt concerning his conclusions. They have, however, such an important bearing on problems in nutrition that it seems very desirable that the question be investigated from all angles[3] and particularly with other animals,[4] and with vitamines

[1] Contribution from the Department of Pharmacology, Harvard Medical School.

[2] *J. Physiol.*, 1912, 44, 425; see also *Biochem, J.*, 1913, 7, 97.

[3] Recently Eddy (*J. Biol. Chem.*, 1920, XXXIV; see also *Am. J. Dis. Children*, 1917, 14, 189), has reported cases of marasmic infants which showed an increase in the utilization of food, when given vitamine extracts prepared from beans.

[4] In another connection the writer has found that mice and rats react differently with certain diets. The question is still open whether or not this difference is qualitative or quantitative.

from different sources. In this connection, it is believed that the procedure to be described later by the writer gives more direct evidence and hence is more free from criticism.

In order to eliminate the question of variability of individual animals from the point of view of efficiency as energy transformers, it appeared to the writer that a better procedure than that used by Hopkins would be to feed a number of animals a basal diet plus such an amount of vitamine-containing material as to keep the animal in weight equilibrium over a period of time. The amount of vitamine would vary with the individual, and would need to be determined in each case. When the animals had been maintained in weight equilibrium over a number of days and the food consumption noted, they could then be fed the same daily ration of a diet containing the same number of calories and having the same composition with the exception of a larger vitamine content and the weight noted. As the greater vitamine content would stimulate the appetite (directly or indirectly), there would be no difficulty about the animals eating the same amount of food as they had eaten in the first stage of the experiment and they would receive the same number of calories and a food with the same gross composition within very narrow limits.

Due to lack of time available for this work, it was impossible, in the preliminary experiment to be described, to bring each animal as near weight equilibrium as was wished, and as it is felt very certain can be done. The average, however, for twelve animals is close and if the results are considered from the statistical point of view, they give further very convincing evidence that the vitamines increases the efficiency of the body in the utilization of the food. The error due to temperature variation it is believed is not large, but this, of course, should have been eliminated. There appears no very easy way of eliminating the error due to greater activity of the animals in the second stage of the experiment. This error may be considerable for there is no question that the animals were markedly more active when given the limited diet with greater vitamine content. The direction of this error makes the results all the more convincing.

EXPERIMENTAL

Preparation of Diets.—The diets were prepared at the beginning of the experiment from the same stock of material and

placed in stoppered containers in the ice box. The composition follows:

	Diet 401	Diet 403	Diet 405
Casein	17.5%	16.5%	15.5%
Starch	49.5%	48.5%	47.5%
Yeast	1.0%	3.0%	5.0%
Lard	18.0%	18.0%	18.0%
Butter	9.0%	9.0%	9.0%
Salts	5.0%	5.0%	5.0%

The yeast contained approximately 0.46 per cent. fat, 46.5 per cent. protein and 38.0 per cent. carbohydrate (32.26 "carbohydrate" plus 5.8 crude fiber). Omitting the negligible quantity of salts the gross composition of the diets was as follows:

	Diet 401	Diet 403	Diet 405
Protein	17.96%	17.88%	17.8 %
Carbohydrate	49.88[5]	49.64[6]	49.4[7]
Fat	27.05%	27.15%	27.25%
Salts	5. %	5. %	5. %

[5] Includes 0.06 per cent. crude fiber.

[6] Includes 0.12 per cent. crude fiber.

[7] Includes 0.3 per cent. crude fiber.

The diets richer in vitamine had slightly lower calorific value.

The *casein* was prepared from the 40-mesh commercial product by shaking up for several hours successively with two portions of 50 per cent. alcohol and one of 95 per cent. alcohol and drying in warm air.

The *starch* was prepared from commercial cooking starch (corn) by the same procedure as for casein.

The *butter* used was the clear fat obtained by decanting the melted butter through a dry filter.

The salt mixture was that described by Osborne and Mendel.[8]

The mice were kept in wire mesh cages under which were pieces of glazed paper to catch feces and wasted food. The food was placed in small salve jars having aluminum covers in which ½ inch holes had been stamped. The jars were placed in 5-inch glass crystallizing dishes during the second stage of the experiment so that any waste food could later be found by the mice and eaten. Almost never in the second stage of the experiment was any food found under the cage. In all cases

[8] *J. Biol. Chem.*, 1919, 37, 572.

the waste was easily separated from the feces and account taken of the amount.

Sixteen mice were fed ten days on a complete diet and then nine days on a diet free of vitamines and then transferred to diet 401 (unlimited amount). After one day on the later intake records were begun.[9] Of the 16 mice started two grew nearly normally on 1 per cent. yeast,[10] one became sick and died, another declined very rapidly. These four were not considered in the experiment. The records of the remaining twelve are shown in the table. They were of varying size and represented somewhat different ages so that the results can hardly be accounted for by the action of the normal intermittent growth impulse.

PERIOD 1. DIET 401 (1 PER CENT. YEAST), UNLIMITED AMOUNT

Animal	Weights			Total Intake	Total Days	Average Daily Intake
	Beginning.	End	Change			
1.....	15.7 gr.	16.62 gr.	+0.92	27.15 gr.	13	2.09 gr.
2.....	11.2	10.49	−0.71	13.61	10	1.36
3.....	14.6	15.31	+0.71	24.75	13	1.9
4.....	11.1	10.45	−0.65	16.53	13	1.27
7.....	12.4	13.0	+0.60	19.83	13	1.52
8.....	14.0	14.97	+0.97	21.19	13	1.63
9.....	15.4	13.68	−1.72	19.39	13	1.49
10.....	14.8	13.71	−1.09	18.25	13	1.4
12.....	14.8	14.69	−0.11	21.91	13	1.68
13.....	18.5	17.05	−1.45	27.85	13	2.14
14.....	10.2	9.12	−1.08	13.77	13	1.06
17.....	15.9	16.58	+0.68	19.97	10	1.997
	168.6	165.67	−2.93	244.20	150	

For 150 mouse days 12 mice ate an average of 1.628 grs. per mouse per day and lost 2.93 grs. or 1.74 per cent. For maintenance, then, they needed slightly more than 1.628 grs. average per day.

Figured from Period No. 1 the mice in the 187 mouse days should have required a little more than 187×1.628 grs. and gained 14.36 grs. or 8.68 per cent.

[9] It would be well to increase this period somewhat.

[10] In general mice require 5 per cent. of yeast in the diet for normal growth; some make substantial gains on 3 per cent. and a very small per cent. require less. The individual variation for rats is, in our experience, considerable though not as great as for mice. The average requirement, too, for mice is markedly more than for rats, the ratio being roughly five for mice to three for rats expressed in per cent. in the diet.

PERIOD NO. 2. DIET 403 AND (OR) 405 (LIMITED)

Animals	Beginnings	End	Change	Total Intake	Total Days	Fed Daily, Grs.
1.....	16.62 gr.	18.74 gr.	+ 2.12	32.64 gr.	16	2.04
2.....	10.49	11.8	+ 1.31	10.95	8	1.4
3.....	15.31	17.03	+ 1.72	30.40	16	1.9
4.....	10.45	11.83	+ 1.38	18.72	16	1.17
7.....	13.00	14.51	+ 1.51	24.00	16	1.5
8.....	14.97	14.05	− 0.92	24.00	16	1.5
9.....	13.68	14.45	+ 0.77	24.00	16	1.5
10.....	13.71	15.25	+ 1.54	24.00	16	1.5
12.....	14.69	14.65	− 0.04	25.6	16	1.6
13.....	17.05	18.84	+ 1.79	35.36	16	2.21
14.....	9.12	9.69	+ 0.57	17.00	16	1.06
17.....	16.58	19.21	+ 2.63	38.00	19	2.00
	165.67	180.05	+14.38	304.67	187	

In order to make more easily comparable the weight changes with food intake in the two periods, table No. 3 is presented.

Animal	Period No. 1			PeriodNo 2		
	Weight Change	Average Daily Food Consumption	Days	Weight Change	Average Daily Food Consumption	Days
1.....	+0.92 gr.	2.09 gr.	13	+2.12 gr.	2.04 gr.	16
2.....	−0.71	1.36	10	+1.31	1.4	8
3.....	+0.71	1.9	13	+1.72	1.9	16
4.....	−0.65	1.27	13	+1.38	1.17	16
7.....	+0.60	1.52	13	+1.51	1.5	16
8.....	+0.97	1.63	13	−0.94	1.5	16
9.....	−1.72	1.49	13	+0.77	1.5	16
10.....	−1.09	1.4	13	+1.54	1.5	16
12.....	−0.11	1.68	13	−0.04	1.6	16
13.....	−1.45	2.14	13	+1.79	2.21	16
14.....	−1.08	1.06	13	+0.57	1.06	16
17.....	+0.68	1.997	10	+2.63	2.00	19

The energy content of the excreta during the two periods was not determined, but Hopkins[11] and Drummond[12] have shown that the energy content of the excreta of rats when fed adequate foods is substantially the same as when fed vitamine-free foods.

The writer regrets the inability to repeat the work with a larger number of animals. It would be desirable to use a vitamine extract, control the temperature of the animal room, get the animals more closely in weight equilibrium by using a vitamine percentage determined by the individual mouse, and to

[11] *J. Physiol.*, 1912, 44, 440.
[12] *Biochem. J.*, 12, 25.

have periods 1 and 2 run over the same number of days. Among the animals there should be included several adults which had been brought to condition of underweight in the preliminary period with vitamine-free foods. This would answer any possible question concerning the effect of the intermittent growth impulse. It is hoped that others may be able to carry on this work.

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R. R. RENSHAW

HARVARD MEDICAL SCHOOL, BOSTON

INTERFERENCE IN PRIMULA SINENSIS

OUTSIDE of *Drosophila*, the only data bearing on the question of interference of crossing over are those which I reported in a paper on linkage in *Primula sinensis*.[1] The phenomenon of interference—our knowledge of which in *Drosophila* dates from crosses made by Sturtevant and analyses made by Muller in 1912—consists of the fact that the occurrence of a crossing over in one region of a chromosome reduces the chances for the occurrence, in that cell, of another crossing over in a different region of the same chromosome; thus there is a smaller number of double crossovers than would otherwise be expected. The amount of interference is expressed by Muller's index called "coincidence," which is the ratio of the proportion of double crossovers actually observed in the experiment to the proportion of double crossovers which would have been expected to occur on the assumption that crossings-over in the two regions were independent of each other; the latter, or "expected" proportion of double crossovers is obtained by simply multiplying together the proportion of crossovers in one region by the proportion of crossovers in the other region. As I stated in my paper on *Primula*, a calculation based upon my total results could not be sufficiently reliable to decide the question of whether or not interference existed in *Primula*. This was on account of an uncertainty in the classification; I now find, however, that a calculation based upon a selected group of the plants, in which the uncertainty does not exist, is sufficient to decide the question in the affirmative—contrary to my earlier conclusion.

Three pairs of genes were involved in the *Primula* crosses—

[1] Altenburg, E., 1916, Linkage in *Primula sinensis*. *Genetics*, 1: 354–366.

those for long style (l), red flower (r), and red stigma (s), allelomorphic respectively to short style (L), magenta flower (R), and green stigma (S). The order of the loci, as based upon 3684 individuals, was l r s; the per cent. of crossovers in the first region (between l and r) was 11.62, and that in the second region (between r and s) was 34.02. These relations, shown in a map, are as follows: $\frac{l}{0}\ \frac{r}{11.62}\ \frac{s}{45.64}$. The per cent. of double crossovers observed in the experiment was 2.52. According to the formula given above, the number of double crossovers to be expected if crossings over were independent would be 11.62 per cent. $\times$ 34.02 per cent., or 4.0 per cent., which, as I noted in the account of the case, exceeds the observed proportion of 2.52 per cent. This difference, then, between the observed and "expected" values in *Primula* would indicate that interference existed here, but, as I further stated, the difference was not significant because of the uncertainty which had attended the classification of flower color in the plants with the gene for green stigma. This gene caused the flower color to be lighter and obliterated somewhat the distinction between red and magenta.

In the plants with red stigma, however, the flower color was dark enough to render entirely certain the classification in regard to red and magenta; these plants, considered alone, would therefore furnish reliable data for determining the interference. I stated that, when these reliable plants alone were taken into account, no evidence of interference was to be found; but this conclusion was due to a numerical error in the calculation of the "expected" number of double crossovers, for I now find, in going over the figures, that the "expected" number is considerably higher than the number observed. Among the 1876 plants with red stigmas, there were 210 crossovers, or 11.2 per cent., in the first region, and 688, or 36.7 per cent., in the second region. The "expected" number of double crossovers is therefore 11.2 per cent. $\times$ 36.6 per cent., or 4.1 per cent. There were 54 double crossovers observed, or 2.9 per cent., giving a coincidence ratio of 2.9 : 4.1, or .7, instead of 1.00, which would be the ratio in the absence of interference. The difference between the "expected" and observed numbers is beyond the limits of random sampling, and it must therefore be concluded that interference exists in *Primula*. Although no reliance can be

placed upon the precise value of the coincidence, it may be noted that this amount, .7, is just what would be expected for a similar distance in the X chromosome of *Drosophila melanogaster* (*ampelophila*).

It should be noted here that Haldane, in referring to my results in a recent article, called attention to the fact that the *Primula* data (using the counts of all classes of plants) fit his formula for expressing the relations between linkage values in *Drosophila*. Inasmuch as any formula expressing the linkage relations in *Drosophila* is necessarily the mathematical resultant of the operation of interference (interference of a type which diminishes with increasing distance), Haldane's statement that the *Primula* data fit the same formula as *Drosophila* is equivalent to saying that interference exists here, as in *Drosophila*; it is in this sense a restatement of my observation that the number of double crossovers found in the total count of the plants is smaller than the number "expected" in a case of a random occurrence of crossing over. It must further be noted that Haldane's formula for expressing the linkage relations in *Primula* is open to the same objection of unreliability as noted above, since his calculation is based upon all classes of plants, instead of upon just those classes which I showed must be used in any reliable determination.

The finding of interference in another organism, so widely separated from *Drosophila*, is of interest because of the bearing of interference on the general theory of linkage. Interference is not accounted for on Trow's form of the reduplication theory, although it was the earlier experiments upon *Primula* itself which largely supplied the data upon which this theory was founded.

EDGAR ALTENBURG

THE RICE INSTITUTE,
HOUSTON, TEXAS.

ON INTERSEXES IN FIDDLER CRABS.

NOT long ago a few specimens of small female fiddler crabs, *Uca pugnax*, were submitted to me by Professor T. H. Morgan for determination. They appeared to be normal, immature individuals and I so stated in my reply. In order to demonstrate the correctness of this view, a loan was made to Professor Morgan from the National Museum collection of a series of imma-

ture female fiddlers showing different widths of abdomen normal to the growing female in passing from the juvenile or immature, to the sexually mature, condition. However, this exhibit apparently had the opposite effect from that intended, as Professor Morgan pronounced them all "intersexes" and thereby seemingly robbed the female fiddler of its period of adolescence.

In his paper "Variations in the Secondary Sexual Characters of the Fiddler Crab"[1] we find Professor Morgan's exposition of the subject. It is not easy to follow the author owing (1) to erroneous or incomplete references to figures and (2) to absence of measurements; for example, "Fig. 4*B*″," cited on p. 225, line 12, does not exist, "Fig. 4*B*′," p. 225, line 3, is cited as a female abdomen when it is really a male, and one can not tell if the two unequal chelæ of Fig. 4*A* belong to one individual, which is probable, and if the two unequal chelæ of Fig. 4*B* belong to one individual, which is probable but not possible, as the text says that they are "of the same size." There are no measurements nor indication of enlargement of figures.

The case under discussion belongs in Professor Morgan's second category of intersexes. He tells us that the specimens are always small, that they are female in character except for the abdomen being narrower than in the mature female, and the abdominal appendages being different from those of the mature female but not at all malelike. Why, one naturally asks, are they not juvenile? His reply is, "because normal individuals of the same size have the abdomen full width." This argument unsupported is fallacious.

Many species of crabs are known to attain sexual maturity at a much smaller size than their maximum and to exhibit considerable range in the size at which they attain that maturity. As an example, two jars full of the common shore-crab of the Pacific coast, *Hemigrapsus nudus*, show egg-bearing crabs ranging in width of dorsum from 10.4 mm. to 32 mm., and among the immature females with narrow abdomens, six individuals which range from 12.5 to 15.7 mm. in width.

Professor Morgan goes on to say that some of the smallest "intersexes" have the narrowest abdomen, that there is no obvious relation between the size of the crab and the relative width of the abdomen, but that there is some correlation between the character of the abdominal appendages and the width of the

[1] AMER. NAT., Vol. LIV, No. 632, May-June, 1920, pp. 220–246.

abdomen. All these point to normal development as the rational explanation.

He figures, p. 226, the abdomens of five female "intersexes," including, I think, but am not sure, two abdomens of successive, or near successive molts in the aquarium. As no two of these abdomens are of the same width, the illustrations would indicate a change in size of body, that is, growth and surely age, with the molt or molts. But Professor Morgan says, p. 225, lines 13–14, "that the condition of the abdomen and claws had not changed."

The fact of the matter is, that neither Professor Morgan nor any one else, so far as I know, is aware of the exact growth changes of our fiddler crabs beyond the first few crab stages. Hyman, in "The Development of *Gelasimus* after Hatching,"[2] carries his painstaking researches only as far as a 4 mm. wide crab stage.

We can at present reason only by analogy from the study of work done on other species of crabs, of which there is altogether too little compared to the facilities offered by the laboratories of our coasts; and such analogy seems to indicate that the crabs upon which Professor Morgan bases his arguments are normal females which had not, in their particular cases, attained sexual maturity. Churchill's "Life History of the Blue Crab"[3] may be cited, and also Pearson's "Cancer. (The Edible Crab.)"[4] Both of these give tables which demonstrate the great variability in the ratio of increase at each act of ecdysis.

It is important, as I have stated elsewhere, that the development of each of our common crabs be carried through from the egg to maturity, that accurate records be made, and properly labeled material upon which such studies are based be deposited in an enduring collection accessible to all who may be interested.

MARY J. RATHBUN

VARIATION IN JUVENILE FIDDLER CRABS

IT is too bad that Miss Rathbun's kindness in sending me specimens from the National Museum "had the opposite effect from that intended." While regretting this, I can only call attention to the fact, stated in my paper, that out of more than

[2] *Jour. Morphol.*, Vol. 33, No. 2, March, 1920.

[3] *Bull. Bur. Fisheries*, XXXVI, November 11, 1919.

[4] *Proc. and Trans. Liverpool Biol. Soc.*, Vol. XXII, 1908.

three thousand individuals that were collected only a few showed the narrow abdomen concerning which Miss Rathbun raises an interesting question. These rare individuals, I ventured to suggest, with some hesitation and with considerable reservations, might be called intersexes, because the variation in question was in the direction of a character peculiar to the opposite sex. Miss Rathbun states that my argument that "they are not juvenile . . . unsupported, is fallacious." Again I can only repeat what was said in my paper, that I examined a very large number of individuals, many of which were of about the same size as the variations in question, some of which were even smaller, and others somewhat larger, and in none of the young females (except in those recorded as exceptions) did I find the abdomen narrow.

May I also recall that I specifically referred to the case of the blue crab in which the abdomen of the juvenile female is narrow, so this condition was known to me, both from the literature and from personal examination, although the reader might gain the opposite opinion from Miss Rathbun's comments.

It is rather strange also that Miss Rathbun neglects to point out that these small crabs with narrow abdomen were stated in my paper to show either a change towards maleness or possibly a retention of the juvenile condition. It is quite possible, of course, that the narrowness of the abdomen of the exceptional individuals might be interpreted as a variation in the direction of the juvenile stage found in other species; but it is certainly not a common stage through which crabs of this size pass. Whether the juvenile interpretation has any advantage over the alternative one that I provisionally suggested can only be settled when we have found out to what this exceptional condition in the fiddler crab is due. My paper was written more with the intention of calling attention to a new and very interesting set of variations in these crabs (as the title indicates) than with the intention of trying to determine what the definition of intersexes shall include; for, as I said, "It seems to me not worth while at present to attempt to classify such material until we have learned more about it."

T. H. MORGAN

[1] Fig. 48′, page 225, line 3, should be Fig. 5*B*′. Fig. 4*B*″, page 225, line 13, should be Fig. 5*C*″. Fig. *A*, *B*, *C*, *D*, page 225, line next to bottom, should be Fig. 5 *A*, *B*, *C*, *D*.

THE TURKEY AS A SUBJECT FOR EXPERIMENT

EXPERIMENTS with our native vertebrates offer many difficulties not encountered when dealing with domestic animals. In the field of genetics especially, while domestic animals continue to furnish enticing problems, it is not strange, therefore, that they practically monopolize the attention of students. No one can foresee how far work of this kind will proceed but it seems probable that some important phases of the subject of variation never can be elucidated by the study of domestic animals alone. Hence it would be very desirable to work with wild forms wherever this is practicable. This would be especially interesting for study of the significance of the intergrading subspecies or "geographic race" which is found so widely in nature but which appears to have no recognizable counterpart in the ordinary variations of domestic animals.

The so-called subspecies perhaps needs no introduction even to biologists who do not have first-hand acquaintance with it, but the extent to which it features in the fauna of the world seems scarcely realized even among those who are quite familiar with it. Within the memory of the present generation, the ultimate division of classification was the species and attempts to divide this into races or varieties were often looked at askance as probably indicating an over-weening desire to multiply names and magnify differences of no phylogenetic significance. In wrestling with the question "What is a species?" many were led to eschew classification entirely and contented themselves with the knowledge that no two individuals were alike and the belief that efforts to associate them were futile. Meanwhile, in spite of headshaking in various quarters, "hair-splitting" has continued until at present nothing is clearer than that the subspecies is a reality constituting a widespread and obvious evidence of active contemporary change in organisms, not only in single individuals but in groups of individuals.

In ornithology and in mammalogy, at least, the old-fashioned species in the vast majority of cases is found to be a composite or a mosaic definitely divisible into units connected by graded series and having a plain relation to geographic distribution. A species of continental distribution in North America, for example, may have one subspecies in the east, one in the north and several in the south and west each occupying a limitable area and each characterized throughout its range by certain features

not possessed by the others. Along the geographic borders of each subspecies will be found specimens showing varying degrees of intergradation so that each form merges with an adjoining one, or, in some cases, one in central position may merge into several others in different directions. If, for any reason, the "areas of intergradation" were rendered uninhabitable, the various subspecies would stand as distinct well-characterized forms presumably until they themselves began to differentiate and separate into parts. Sometimes the difference between recognizable subspecies is slight, or sometimes it is very marked, but when gradations through several forms are followed, characters are almost always found to change to a degree far beyond any probability of an ontogenetic explanation. It may be emphasized that subspecies of this sort are not the exception, but the rule. It might almost be said that the existence of diverse inosculating units correlated with geography is characteristic of terrestrial vertebrates. Continued study with improved facilities and increasingly comprehensive collections from all parts of the world constantly reduces the number of forms which are not known to break up into subspecies. To a very great extent, the presence of an undivided 'full species" in our checklists signifies either that it is a senescent type of limited distribution or that, for lack of material or opportunity, it has not been studied intensively. The intergrading subspecies has not been recognized so widely among invertebrates nor in plants, but neither entomologists nor botanists have collected and studied their material from the geographic standpoint to such an extent as the ornithologists and mammalogists, so it cannot be said that the process of change illustrated by the subspecies is not even more widespread than appears from the study of birds and mammals.

The process of formation of these subspecies, therefore, is going on before our very eyes in wholesale fashion and it is difficult to believe that it is, as someone has said, merely a "shuffling of the cards" which in the long run means nothing to evolutionary progress. Rather does it seem that it must have a physiological basis, a relation to germinal change, and a large potentiality for affecting the general course of evolution. Despite its evident importance, the intergrading subspecies is receiving but scant attention from experimental zoologists. With the conspicious exception of the very significant work being done with

white-footed mice by Dr. F. B. Sumner of the Scripps Institution,[1] there seems to be little or no work under way which can be correlated logically with the results of speciation and subspeciation as the field naturalist and taxonomist find them in nature. Doubtless one of the principal reasons for this is the difficulty of finding convenient subjects and suitable conditions for such work. Perhaps another is the independence of workers in the respective fields of taxonomy and experimental zoology.

As promising subjects for experiment, it seems worth while to call attention to the American wild and domestic turkeys. The common turkey has an exceedingly desirable distinction from other domestic animals in that there is no important question as to its history and lineage. Moreover, the wild stock from which it was derived represents one of several intergrading subspecies the natural characters and relationships of which can be determined with a great degree of accuracy. Hence our Thanksgiving bird, as a subject for experimental breeding, might furnish a combination with which naturally and artificially induced characters could be studied comparatively. As at present recognized and understood, the native American turkey is divisible into six races or subspecies, as follows: One from the southeastern United States (*Meleagris gallopavo sylvestris*); one from southern Florida (*M. g. osceola*); one from central Texas and northeastern Mexico (*M. g. intermedia*); one from Arizona, New Mexico and Chihuahua (*M. g. merriami*); one from the Sierra Madre of Jalisco and west central Mexico (*M. g. mexicana*); and one from the eastern cordillera of Vera Cruz, Mexico (*M. g. gallopavo*). The range of the turkey group is thus from the southeastern Atlantic seaboard westward to the Rocky Mountains and thence south to Vera Cruz. Complete intergradation between the various subspecies may not be demonstrable with absolute nicety in all cases because the birds were exterminated in certain parts of the range before any specimens were preserved. That intergradation between all the races was as uninterrupted as it can be shown to be between some of them, however, is beyond reasonable doubt. The extremes of differentiation, as usual in such cases, are represented approximately by the geographical extremes. The characters distinguishing the wild turkey of the eastern United States from that of south-

[1] See especially AM. NATURALIST, XLIX, pp. 688–701; *ibid.*, LII, pp. 177–454, 1918.

ern Mexico, therefore, are clear cut and readily recognizable without the application of any greatly refined methods. The obvious distinction is found in the feathers of the tail and upper tail coverts which in the United States bird are broadly tipped with rich chestnut whereas in the Mexican subspecies these parts are white or nearly white. Such characters, in animals of unknown history, might easily be looked upon as produced by mutation; but with complete gradation from one to the other known to exist in nature, it is hard, at least for some of us, to believe that the difference was not accomplished by gradual rather than sudden change. If it could be shown that characters of this kind behave as hereditary units without any such blending as requires "dialectic gymnastics" to explain, it would be a long step forward in the correlation of natural and man-made experiments. Such characters are in fact heritable, as has been shown by Sumner in his breeding and transference experiments with *Peromyscus*. This is illustrated also by an undirected experiment to call attention to which is one of the objects of this communication, namely, the test of subspecific characters which has been carried out in the domestication of the turkey.

As is widely known to sportsmen, breeders, and many others, our domestic, so-called bronze, turkey is readily distinguished from the wild bird of the eastern United States by the coloration of the upper tail coverts and tail. The reason for this, which is not so generally known, is not that the domestic bird has changed in these respects under man's influence, but because it is the direct descendant of the Mexican wild race which differs from the northeastern race by these selfsame characters. Carried from Mexico to Europe in the early sixteenth century and thence brought to the United States, it has continued for more than three hundred generations in a new environment maintaining its old established subspecific characters. To-day it may differ from the Mexican wild race in some details, but its general coloration is the same and especially does it retain its taxonomically diagnostic features. These, therefore, are heritable and doubtless related to germinal conditions which became fixed in the wild bird. Since the characters themselves are of the kind that appear to be produced by insensible gradations and of the kind that frequently bear an obvious relation to environment. an easy deduction would be that the germ plasm also has

changed gradually and, at least as a working hypothesis, one might suppose that the germ plasm had been affected directly or indirectly by the environment. Such an explanation is, of course, far too simple and old-fashioned for present-day students of evolution. It does not explain a multitude of undeniably important and fascinating results of experimental work. But neither do theories of mutation and the maze of modern genetics explain the intergrading subspecies and perhaps there is room for at least a little experimental work which does not deny such an hypothesis at the outset.

In the case of the turkey, while the relatively inconspicuous subspecific character has proved itself stable, violent saltatory changes have been established easily. These are of the sort common among close bred domesticated animals but so rare among wild vertebrates that no one has yet found a case in which they can be shown to have been perpetuated by natural process. Thus we now have self-colored breeds of turkeys respectively black, white, buff, and blue gray as well as the breed called Narragansett in which the feathers are tipped with steel gray. Hence it seems that the turkey may offer an opportunity for comparative study of the hereditary behavior of characters which have developed naturally by what seems to be continuous variation and those which have appeared discontinuously and been perpetuated artificially. Sumner (l. c., 1918) has found with *Peromyscus* that hybridization of different subspecies produces in the F_1 and F_2 generations a blending of the subspecific characters comparable to the gradations found in nature, whereas mutant characters (partial albinism, etc.) act as simple Mendelian units. In other words, natural subspecific characters act in hybridization experiments as they would be expected to do on the assumption that they were produced by continuous variation. Whether or not the same results would follow with the turkey and other forms would seem to be well worth determining. In a general way, it is known to breeders that hybrids between wild and domestic turkeys are of intermediate type, but so far as I know careful well-controlled work has not been done.

WILFRED H. OSGOOD

FIELD MUSEUM OF NATURAL HISTORY

THE "ONE-LETTER" RULE FOR GENERIC NAMES IN ZOOLOGY

RULES of nomenclature as they affect scientific names in zoology would no doubt serve their real purpose best and give more general satisfaction if used, not in an absolute sense, but with discretion. But there are those to whom a rule is a rule to be rigidly applied, and the results are such that the question is raised whether if we must abide by rules, we cannot have better ones. A nomenclatorial rule used by the American Ornithologists' Union has raised this question in the mind of the writer, and attention is here called to it, not in a controversial way, but merely to insure that the other side of the case is presented. If such questions are ever taken up again by an International Commission it is desirable that data and opinions on the vexed points be available for consideration. In the recently published[1] "Seventeenth Supplement to the American Ornithologists' Union Check List of North American Birds," prepared by the Committee on Nomenclature, we find the following statements relating to certain generic names:

Oxyura Bonaparte 1828 is considered preoccupied by *Oxyurus* Swainson 1827 (p. 446).

Nyctala Brehm, from whatever date taken, is preoccupied by *Nyctalus* Bowditch 1825, and *Ægolius* Kaup, 1829, is preoccupied by *Ægolia* Billberg, 1820 (p. 447).

Bucephala Baird 1858 is preoccupied by *Bucephalus* Baer 1827 (p. 446).

Dendrocopos Koch, July, 1876, is preoccupied by *Dendrocopus* Vieillot, April, 1876 (p. 448).

But

Heteroscelus Baird 1858 is not invalidated by *Heteroscelis* Latreille 1825 (p. 443).

Tyto Billberg (1828) is not preoccupied by *Tyta* Billberg (1820) (p. 447).

and

Moris Leach . . . adopted because considered neither a *nomen nudum* nor preoccupied by *Morum* Bolten, although *Morus* Vieillot . . . having a termination differing merely in grammatical gender from *Morum* Bolten is thereby invalidated (p. 441).

[1] *The Auk*, Vol. 37, No. 3, July, 1920, pp. 439–449.

Even the experienced taxonomist might be greatly puzzled by this collection of apparently inconsistent assertions, did he not turn to the Code of Nomenclature of the American Ornithologists' Union (1908 Edition) and find the following explanatory remark under Canon XXX:

> Generic and specific names . . . are to be considered identical . . . whether the ending is masculine, feminine or neuter or in Greek or Latin form.

In the principal codes of zoological nomenclature the practise called for by this rule is sanctioned only by that of the American Ornithologists' Union. The parent (we may say) of the A. O. U. Code, namely the Stricklandian Code, in so far as it touches on the point, would seem to accept very similar generic names, even those differing by only one letter. Section 10[2] says

> A name should be changed which has before been proposed for some other genus in zoology or botany.

This section is further elaborated as follows:

> By Rule 10 it was laid down, that when a name is introduced which is identical with one previously used, the latter one should be changed. Some authors have extended the same principle to cases where the later name, when correctly written, only approaches in form, without wholly coinciding with the earlier. We do not, however, think it advisable to make this law imperative, first, because of the vast extent of our nomenclature, which renders it highly difficult to find a name which shall not bear more or less resemblance in sound to some other;[3] and, secondly, because of the impossibility of fixing a limit to the degree of approximation beyond which such a law should cease to operate. We content ourselves, therefore, with putting forth this proposition merely as a recommendation to naturalists, in selecting generic names, to avoid such as too closely approximate words already adopted (p. 118).

These provisions were adopted (with a reservation as to botanical names) by the British Association for the Advancement of Science in 1865 as part of a code which more than any other guided the course of subsequent nomenclature practice.

[2] Rep. British A. A. S., 1842 (1843), p. 113.

[3] If this was true in 1842, how much more difficult the situation must be now after 80 additional years of taxonomic activity.

In Dall's "Discussion of the Subject of Nomenclature" of 1877 which was based on a circular responded to by 45 American naturalists in addition to previous codes and other publications on the subject, the point under consideration receives the following attention in Section 65, Paragraph 10,

When a name is identical, when properly spelled according to a derivation given by its author, with a prior valid name in the same kingdom it must be rejected.[4]

In other words, if names are not *identical* they stand.

The Entomological Code (1912, Paragraph 82) has this to say on the subject:

A generic or subgeneric name is a homonym and subject to replacement when it is spelled exactly like a previous valid generic or subgeneric name, letter for letter. However, I and J, and Eu and Ev at the beginning of a name are considered the same, and other words that are equivalent in established Latin usage.

In extracts from a code of Nomenclature in Ichthyology (Jordan, Evermann and Gilbert) published in the Condor in 1905, Canon XVII (Second paragraph), is quoted as follows:

As a name is a word without necessary meaning, and as the names are identified by their orthography, a generic name (typographical errors corrected) is distinct from all others not spelled in exactly the same way. Questions of etymology are not pertinent in case of adoption or rejection of names deemed preoccupied. (Note.) This canon prohibits change of names because prior names of similar sound or etymology exist. It permits the use of generic names of like origin but of different genders or termination to remain tenable.

The International Code which, so far as it goes, is adhered to by a majority of zoologists, alludes to this subject in a recommendation under article 36. The language follows:

It is well to avoid the introduction of new generic names which differ from generic names already in use only in termination or in a slight variation in spelling which might lead to confusion. But when once introduced, such names are not to be rejected on this account.

[4] Nomenclature in Zoology and Botany, Salem, Mass., December, 1877, p. 49.

Opinions 25 and 34 of the International Commission support the wording of the foregoing recommendation which is referred to in the opinions as an effective part of the code.

Thus zoological codes in general support the so-called "one-letter rule." The point in this connection that appeals to the present writer with special force is that there would seem to be no good defense for the practise of rejecting names differing in terminations expressing gender and at the same time accepting other names differing by no greater margin (often by only one letter).

Thus under A. O. U. practise *Otostomus*, *Otostoma* and *Otostomum* are treated as identical, while *Odostoma* and *Otostoma*, *Icteria* and *Icterias*, *Pica* and *Picus* are considered distinct. The fact that the latter words had different terminations, or different meanings in classical usage has nothing to do with the case. Nomenclature is not the Latin language; it is a mass of invented, adopted, derived and compounded words, some of which are in Latin form, others not, but all of which, nevertheless, have equal standing in the scientific world. Principle V of the A. O. U. Code, itself, asserts that

> A name is only a name, having no meaning until invested with one by being used as the handle of a fact; and the meaning of a name so used in zoological nomenclature, does not depend upon its signification in any other connection.

Literally construed this principle is fully in accord with the definition of scientific names as arbitrary combinations of letters, and it would seem unnecessary even to state with respect to arbitrary combinations, that we can only regard each different one (even if by only one letter) as a distinct name. It would seem clear, therefore, that in scientific nomenclature names are merely labels for conceptions; that their use demands precision, and with precision all names appreciably different can be used without confusion.

Small (even one-letter) differences in scientific names are by no means confined to terminations; they occur in all points in words. Consider: *Neothripa, Neothrips; Felicea, Felicia, Donatia, Donacia; Isotoma, Isosoma; Leptopora, Leptoprora; Mercera, Merciera; Teliocrinus, Teleiocrinus; Sciurus, Seiurus; Sus, Mus.* Consider also such a series of names as *Monocerus, Monocereus,*

Monocercus, Monocercis. These names all stand under the A. O. U. Code, as do also words like the following: *Rolanda, Rolandra; Oga, Ogoas; Orophia, Orophila; Menida, Menidia; Lyria, Lyrcia; Passerina, Passerita;* all of which differ only in the last few letters as do those with terminations denoting gender, and are equally liable to confusion by typographical errors.

What justification is there for accepting names so nearly alike as many of these but considering as homonyms such terms as *Nyctala* and *Nyctalus; Nettion* and *Nettium?* The aim of codes of nomenclature is to conserve names, not to make opportunities for the creation of new ones. But the A. O. U. custom of considering homonyms, names differing in terminations indicating gender is a breeder of new names. This is clearly shown by two notes[5] published in a recent number of *The Auk*, in which it is asserted that *Phæochroa* Gould 1861 is preoccupied by *Phæochrous* Laporte, 1840, and *Elminia* Bonaparte 1854 by *Elminius* King, 1831, and a new name is proposed in each case. The same criticism applies to certain other suggestions in connection with Canon XXX of the A. O. U. Code, namely those that would homonymize such words as *Athene* and *Athena; Contopus* and *Contipus.* Those who look with favor on homonymizing words whether they differ only by endings denoting gender, whether the root is taken from the Attic or other dialect, whether the connecting vowel of compound words be a, i, or o, or for other philological reasons should remember that there is no more reason for stopping at one point than another in the path of purism. Always there will be more and more advanced purists, who would sink generic names differing far more widely.[6] For instance, consider the following pairs of names for which it has actually been proposed that the second name in each couplet be regarded as homonym: *Callitriche, Calothrix; Myosuros, Myurus; Galarhoeus, Galactorheus; Korycarpus, Corythrocarpus; Ionactis, Iactis; Genyscoelus, Coelogenus.*

Philology is an interesting and important science, but what has classical purism to do with a hodge-podge of names such as zoological nomenclature now is, with names coined, with names classical, with those borrowed from nearly every language ancient and modern? What would be the fate of nomenclature if the purist were allowed to work his will with such names as: *Abudef-*

[5] *The Auk*, Vol. 37, No. 2, April, 1920, p. 295, and p. 302.

[6] A very few reject words of similar sound—phononyms.

duf, Avahi, Aye-aye, Bagre, Cachalot, Djabub, Grysbock, Jafar, Jukaruka, Kahavalu, Louti, Mabuya, Maki, Ompok, Potto, Sandat, Sheltopusik, Tlja, Susu, Wallago, Zingel and the like? Or with such personal and local derivatives as: *Amiskwia, Ernestokokenia, Ischikauia, Mitsukurina, Mordwilkoja, Schlaginhaufenia, Takakkawia, Wankowiczium, Wlassicsia and Zschokkeella?*

The writer does not defend the choice of such names, but once on record they are an integral part of nomenclature and an outburst of purism sufficient to do away with them will not occur. Whether we will or no, we are dealing with essentially arbitrary combinations of letters arbitrarily selected. The conglomeration of generic names in zoology, may be, nay is, subject to criticism, but it exists, is in use. It is part and parcel of the language of Science and classical purism can no more be applied to it than to any other modern language which is constantly growing, ever adding to itself terms from a multitude of sources.[7] A condition not a theory confronts us; practicability must reign and pedantry be forgotten.

Practically all rules relating to the validity and priority of generic names have some saving clause as "typographical errors corrected," or "except for obvious typographical errors." A common-sense application of such clauses would do away with the most vexatious cases of emendation, cases often cited to show the necessity of homonymizing similar generic names, namely those in which an author mis-spells names of his own establishing when using them subsequently to the original citation. In such cases why can we not take an author at his word; he intended to treat of the same group as before, and his emended name, whether intentional or not should be regarded as a synonym of the original. We do not recognize an author's efforts to change a published name, except to correct typographical errors. Why should we give any weight to emendations which themselves, in many if not most cases, are almost certainly typographical errors. The same rule should apply to names mis-spelled by others than the original author when it is clear they intended to refer to the same genus. The fact that the species included under such names are now considered to belong to different genera is of no consequence; these genera

[7] Thus we adopt into English but do not Anglify such words as hangar, machete, fez, mufti, a host of which could be cited.

should date from the time formally recognized and should bear the name then given. It is a travesty on priority to credit an author with conceptions he never entertained, and to use for them mis-spelled names for which he no doubt often had occasion to regret his inadvertence. In brief, regard all emendations as typographical errors unless there is definite evidence to the contrary. With the treatment suggested, such cases as *Pogonius, Pogonias, Pogonia* (a name spelled three ways in the same publication), and similar instances lose their troublesome aspect, and suggestions for homonymizing them, much of their force. The chief cause for anxiety in connection with the one-letter rule seems to be that numerous emendations may be revived, but it can confidently be asserted that, from a practical viewpoint, most emendations are clear synonyms from the beginning and their status would not be changed under the one-letter rule.

Moreover changes under this rule need be feared only in branches of zoology in which the practice advised by the A. O. U. Code has been followed, that is the study of birds and mammals. Certainly the one-letter rule has been used, since the adoption of the International Code, if not before, by most American students of animal parasites,[8] echinoderms, crustaceans, insects and fishes[9] and as shown in preceding paragraphs their practice[10] in this respect is overwhelmingly supported by the various zoological codes.

[8] See discussions by Ch. Wardell Stiles (*Zool. Jahrb.*, 15, 1902, pp. 172–175). "The difference of a single letter, entirely regardless of the etymology, excludes the possibility of the words being identical, hence excludes the possibility of their being homonyms" (pp. 172–173).

[9] See note in Jordan and Evermann, "The Fishes of North and Middle America," Vol. I, 1896, p. v, "We regard all generic names as different unless originally spelled alike."

[10] An attempt to develop what usage, in this respect, is followed in a larger number of zoological specialties, was made by mailing a brief questionnaire to 30 systematic zoologists. The questions asked were:

1. In your specialty have one-letter differences been regarded in recent years (at least since adoption of International Code) as sufficient to establish the distinctness and validity of generic names?

2. Or has the ruling of American Ornithologists' Union Code relating to homonymizing terms differing only in endings indicating gender, etc., been followed?

Only 17 replies were received, of which 2 were noncommital, 9 reported no established usage and those which indicated adherence to one or the other of the opposed practise numbered 3 in each case. The result of this mail test at least supports the writer's contention that the subject is one ripe for public discussion.

Since one-letter differences in generic names are sufficient in many cases as shown by citations in this article and the practise of nomenclators, why are they not in all? The one-letter rule is practicable, while one based on grounds of classical purism is not, and as the framers of the Ichthyological Code properly remark:

> If all names are regarded as different unless spelled alike, these matters offer no difficulty. Any other view gives no assurance of stability.

Finally, discarding names of independent origin and distinct application, that are not spelled identically, overthrows the law of priority and like all practises of that tendency (so long as the priority system is followed) is not for the lasting good of scientific nomenclature.

W. L. McATEE

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IMMUNE SERA AND CERTAIN BIOLOGICAL PROBLEMS[1]

PROFESSOR M. F. GUYER

DEPARTMENT OF ZOOLOGY, UNIVERSITY OF WISCONSIN

Mr. President, Members of the Academy of Medicine of Cincinnati: I am deeply appreciative of the honor conferred upon me by the invitation to address this medical society. Although my own researches have lain outside the conventional limits of medicine, it happens that several of them have crossed the border lines of this science and have thereby quickened the naturally keen interest in the scientific aspects of medicine that I have always entertained. In my present researches, indeed, I have borrowed some of my most important tools and ideas from the field of immunological studies and discoveries, made in the main by medical researchers. The luxuriant growth of literature in recent years on immunity, antitoxins, cytotoxins, bacteriolysins, hemolysins, opsonins, precipitins, agglutinins, anaphylaxis, and what not, is known to you all. Naturally, the brilliant series of practical applications of this new knowledge in diagnosis, prophylaxis and therapeutics, stimulated every medical investigator to redoubled effort until the field has become almost exclusively the domain of the bacteriologist and the pathologist.

It may seem presumptuous of me, a biologist, to step outside the traditional bounds of my science and to come, carrying coals to Newcastle as it were, in recounting to you various facts long since learned by physicians—

[1] An address delivered before the Academy of Medicine of Cincinnati.

facts which lie at the very foundation of modern medical theory and practice. I offer my apology in advance for the lack of novelty in much of what I shall say. My only justification is that a reconsideration of such familiar knowledge gives one a good running start, so to speak, for a leap into less known realms; realms of great interest to the embryologist, the cytologist, the student of heredity and of evolution; regions in which lie hidden the secrets of all life and form, of hereditary transmission, and of its converse, variation.

It is clear that the phenomena which constitute the field of immunology, although to-day viewed mainly from the standpoint of infection and immunity, all have broader biological aspects. They must in last analysis be but heightened or specialized reactions of the fundamental processes which underlie all life phenomena. They are but one of the many expressions of that delicately balanced stereochemical system we call protoplasm, and they are inextricably interwoven in the ebb and flow of metabolism, with such fundamental biologic processes as growth, reproduction, irritability and adaptation.

The physiologically minded biologist also inevitably suspects close relationship between the reactions described by the serologist and those manifested normally in a living animal by that wonderful system of chemical messengers or internal secretions, the hormones and chalones, which, independently of the nervous reflex, can stimulate or inhibit the activity of some organ in a part of the body far distant from the source of the secretion itself, and which undoubtedly play an important part in development. There seems no reason to doubt that both hormones and antibodies, for example, represent complexes of atoms which were originally parts of body-cells concerned in the normal metabolic processes. One is extruded into the body fluids under the influence of a usual and therefore normal stimulus, the other is the product of an accidental stimulus resulting from disease or other unusual condition.

In any event this whole field of endocrinology and serology stands as a perpetual challenge to the experimental biologist. Some sixteen years ago Nuttall published his remarkable series of studies on "Blood Immunity and Blood Relationship" in which he reported the results of his examination of some nine hundred different samples of blood from various kinds of animals. He demonstrated that by the precipitin test a differential scale of actual blood relationships among animals can be established. As you doubtless recall, when an animal of one species is injected parenterally with successive doses of blood-serum of another species over a period of a few weeks, the blood-serum of the injected animal acquires the ability to form a precipitate with that of the alien species when the two sera are mixed. When the reaction is carried on in vitro, even in dilute solutions, the cloudiness and ultimate flocculation which results are easily seen. If, for example, a rabbit is thus repeatedly injected with human blood its blood-serum when mixed with slightly diluted human blood-serum in vitro will almost instantly yield a noticeable precipitate, though a control mixture of human blood-serum and the blood-serum of an untreated rabbit will remain clear. The ingredient which has been engendered in the serum of the rabbit is termed a *precipitin*, and the foreign serum which was injected—human blood-serum in this case—is called the *antigen*, or more specifically, the *precipitinogen*. It is known that not only blood-serum, but also milk, globulins, various albumins and bacterial products—in fact probably any foreign protein—may serve as antigen for the formation of precipitins. The reaction is not absolutely specific in low dilutions since species of animals related to the one from which the antigen was taken will also, though in less degree, give the effect. Closeness of relationship is determined by finding the dilution in which the serum to be tested will react. For instance, Nuttall found that when rabbit serum which has been sensitized against human serum is mixed with the moderately di-

luted sera of man, apes and monkeys, respectively, it reacts to all, though in a varying degree. When mixed with more highly diluted sera from such animals, it forms a precipitate only with the serum of man and the manlike apes (chimpanzee, orang-outang, gorilla), the chimpanzee standing nearest to man. Absolute specificity may be obtained if the antigen is sufficiently diluted. On the basis of extensive experience, Uhlenhuth sets a dilution of antigen of 1 to 1,000 as a standard beyond which no precipitation will occur except with the specific antigen employed in the sensitization.

Thus the precipitin test became useful to the zoologist in discriminating between different species, and it may prove of importance in establishing the taxonomic position of new forms, or in confirming or changing the classification of groups already known. The delicacy of the test is remarkable. A properly sensitized serum may give a reaction with blood diluted 20,000 or even 50,000 times. Sera have been obtained, indeed, in which specific antigen could be detected in a dilution of 100,000. When one recalls that ordinary chemical tests cease to give detectable reactions in blood diluted 1,000 times, he can appreciate the value of these physiological methods of measurement to the biologist. They apprise him of species differences between the proteins of various animals which can not be determined by any known chemical methods.

The value of the precipitin test in forensic medicine, in determining the nature of blood stains on clothing, weapons or other objects, is well known to all of you, as is doubtless their utilization in meat inspection, such as for the detection of horse-flesh or dog-flesh in sausages or other chopped meats, and in various other types of adulteration.

One thing that interests the biologists greatly in the precipitin reactions is the fact of so-called "species specificity"—the fact that blood sensitized against one tissue of a given foreign species will react with extracts of

the other tissues of that species. Thus the blood-serum of a rabbit which has been treated with sheep blood-serum will form a precipitate not only with the sheep serum, but with the extracts of sheep muscle, sheep liver, sheep spleen, and other organs of the sheep. This clearly implies that each species of animal possesses something in common throughout all its tissue proteins, something peculiar to that particular species which in last analysis must be resolved into a problem of its general metabolism and stereochemistry. This does not mean that organs may not also have protein complexes peculiar to themselves. Indeed, it is an established fact that they do. And what is more, some of these organal peculiarities may be common to various species. For example, the fact of "organ specificity" has been established for the crystalline lens. According to Uhlenhuth, immunization with crystalline lens of a given species of animal yields a precipitin which reacts with the lens proteins of many different species of animals. Von Dungern and others have secured similar results with proteins derived from the testis. Confirmatory evidence of this fact that a type of specificity attaches to the nature of the organ itself, irrespective of species, has also been established by means of the reaction of anaphylaxis.

The precipitin reactions, then, teach the biologist that in the chemistry of the general proteins of a given animal, there are certain fundamental similarities, also that there are constant species differences between the homologous proteins of different species of animals, and lastly, that some proteins, in certain highly specialized organs at least, though existing in different species, possess similar chemical characteristics.

These and related facts when considered in conjunction with such as those of Reichert and Brown regarding the stereochemical correspondences in the living matter of allied species as demonstrated in the crystallography of their hemoglobins, or the studies of Reichert on the relations of the starches and tissues of parent-stocks to those

of hybrid-stocks in plants—such facts taken all together are gradually constructing for the biologist a rational biochemic basis for the study of the fundamental processes operative in metabolism, heredity and evolution.

But let us now turn our attention to another type of serological reactions, those concerned with the cytotoxins or cytolysins. You doubtless all recall the well-known experiments of Bordet, in 1898, in which he found that the blood of guinea-pigs which have been repeatedly injected with the red blood corpuscles of the rabbit, acquire the property of rapidly dissolving rabbit corpuscles. This is the familiar phenomenon of *hemolysis*, and the substance in the blood-serum of the guinea-pig which brings about solution of the red corpuscles of the rabbit is termed a *hemolysin*. Bordet showed further that this enhanced solvent action of the serum of animals treated with the red blood cells of a different species exists only for the kind of red corpuscles used as antigen, not for those of other species of animals. Exceptions occur, though in the main the reaction is specific. The similar facts regarding bacteriolysins, which are now commonplaces of every-day medicine, had been established a year earlier.

It was soon discovered that other materials such as leucocytes, nervous tissue, spermatozoa and crystalline lens, when injected into the blood of a foreign species will form lytic or toxic substances more or less specific for the antigen used in the immunizing process. While it is probable that none of such cytotoxins or cytolysins acts only upon its own antigen—all studied so far have been found to be somewhat hemolytic—the important fact, for our present purposes, is that although a particular cytolytic serum may affect some other tissues, it vigorously attacks the special tissue used as antigen.

This fact, when fully grasped, suggests inevitably to the biologist, or at least to the investigator interested in the mechanism of heredity and variation, queries such as the following: if a special serum can thus be constructed

which will single out and destroy a certain element of an adult organism, is it not possible that there is sufficient constitutional identity between the mature substance of that element and its representatives in the germ-cell that they too will be influenced? Is this not a way of getting at the old yet ever new problem of the inheritance of body acquirements, or at least of breaking in on the germ? Is it not possible to secure selective action on certain parts of the developing embryo and thus shed some light on the genesis of congenital abnormalities? And by using the cytolytic and other immunologic methods may not additional knowledge be gained concerning the relations of mother and fetus?

Of this series of problems the one which tantalizes the biologist most of all, perhaps, is that concerned with the possible hereditary transmission of characters acquired directly by the body of a parent. As you know, this has been a bone of contention for many years. The so-called Neo-Lamarckians follow, at least in a modified form, the teachings of Lamarck to the effect that such "acquired characters" are or may be inherited; the other school, often called Neo-Darwinians, strenuously deny such inheritance, and assert that the sole font of specific change lies in the germplasm. According to them any new inheritable feature which appears first arises in the germ and only finds somatic expression when this germ develops into a body.

How important he considered the correct solution of this problem is shown in the following statement of Herbert Spencer. He said: "Concerning the width and depth of the effects which the acceptance or non-acceptance of one or the other of these hypotheses must have on our views of life, the question, Which of them is true? demands beyond all other questions whatever the attention of scientific men. A grave responsibility rests on biologists in respect of the general question, since wrong answers lead, among other effects, to wrong belief about social affairs and to disastrous social actions."

Lamarckism at the present time, among American biologists, has all but disappeared. Some palæontologists, who in reading the records of the past find that whenever new conditions for existence occurred, new forms of life admirably adapted to those conditions appeared, are prone to believe that the environment has in some way directly molded these new inhabitants to its bounds. Since this performance has occurred again and again, they are a bit skeptical of the selectionist tenant that each occasion has had to await, not only the accidental occurrence of a favorable germinal variation, but of a host of them, which must in turn be sifted and parceled and perfected by natural selection into that adaptedness to the surroundings which characterized the organisms in question. Various students of geographical distribution also are inclined to regard the direct action of environment as instrumental in molding the fauna of a given region. In brief, those who look at the problems of evolution from wide perspective tend to postulate that altered function or environment, if long continued, in some way modifies descendants, but they don't tell us how. Those who view the problem from the standpoint of the few generations intensively studied by the geneticist, or from the germ-cell lineages of the embryologist, or the chromosomes of the cytologist, almost without exception reject the Lamarckian interpretation. And it can not be denied that the latter have an incomparable advantage in directly testing the matter, since they have their material in hand for direct observation or experimental control. So it has come about that the believer in Lamarckism, silenced if not convinced by the formidable array of negative evidence amassed against him, and still more perhaps by his own inability, from the basis of carefully controlled experiments, to cite specific examples of inherited somatic acquirements, has subsided into mute acquiescence or but faint-hearted advocacy of his theory.

The fertilized egg develops into an adult individual

through a series of cell-divisions and specializations of the new cells thus produced. During development certain cells are set apart, often very early in embryogeny, for reproducing the next generation. Thus the germ-cells and the body-cells of a given organism develop at the same time and neither is the product of the other; each alike has originated by division from the fertilized ovum. There is no necessity, therefore, for collecting samples from all parts of the body and concentrating them in germ-cells, as Darwin supposed was done, for the samples are already there, derived from the same supply that produced the parental body. They exist not in the form of such parts of an organism as are visible to us, but simply as certain ingredients which when combined in certain ways and developed in certain directions give rise to the parts in question. Sooner or later the body dies, but in the meantime one or more of the germ-cells have passed on to become expressed as new bodies and new germs. Thus a child does not inherit its characteristics from corresponding characters in the parent-body, but parent and child are alike because they are products of the same fundamental materials.

How, indeed, can a change in a brain-cell or a muscle-cell find expression in a germ which is itself a cell that possesses neither brain nor muscle? How can an influence at a distant part of the body even reach a germ-cell? How can immature young, even larvæ in some instances, produce young which ultimately come to manifest the characteristics of the adults of the species? How can recessive Mendelian unit-characters disappear, perhaps for generations, to reappear at last apparently with qualities undimmed? How, on the Lamarckian basis of use-inheritance, can the highly specialized characters of the worker-bee have originated and become perfected when the individual itself is sterile? How account for adaptive characters based on passivity, or for mutual adaptations such as may exist between plants and certain animals? These and a host of questions like them con-

front the Lamarckian when he strives to resuscitate the faith that is in him.

The opponent of Lamarckism certainly shines as a disconcerting questioner. Moreover, he is clearly correct in his contention that the idea of germinal continuity is the simpler one, and probably the only tenable one, as regards the inheritance of characters, *once they have been engendered.* But the crux of the whole problem lies in the question, where do new characters come from? According to the followers of the great biological theorist, Weismann, not only do new heritable characters originate in the germ, but a change which first appears in the body can not in any way become incorporated in the germplasm. Unquestionably, constitutional changes in a germ-cell at any time may find expression as a new or modified character in the subsequent organism which comes from this germ. But while this is an obvious fact, it gives no real explanation of the origin of the character in question, since it tells us nothing about what induced the constitutional change. Weismann regarded sexual reproduction, the intermingling of two lines of germplasm, as an important cause of germinal variation, but our modern genetical studies indicate that this is probably not true. Dual ancestry, of course, makes possible new arrangements of germinal constituents which reveal themselves in new combinations of characters, but the germinal antecedents of such combinations are unitary in nature, and there is no evidence that sexual mixture originates any new units. So the Neo-Darwinian, although highly successful in pointing out the shortcomings of Lamarck, has been little if any more successful in explaining satisfactorily how changes are *initiated* in the germ-cell. Yet it is this very item of change, of *variation,* that is the real basis of evolution.

Some selectionists glibly assert that new characters arise as the result of spontaneous changes in the germ. What is meant by this? Just what is a spontaneous change? No one has ever succeeded in telling us. And

we may suspect, though perhaps it is heresy to do so, that it is a well-sounding phrase that is the equivalent of the three words, I don't know. Unwilling to admit of the modifying influence of external agencies on the germ, such theorists resort to the fiction of a spontaneous change. Coleridge somewhere has said "What's gray with age becomes religion." We have toyed so long with this idea of germinal continuity and the invulnerability of the germ, that it has become for some of us well nigh sacrosanct. Living matter is living matter wherever it may be found, but when it happens to be in the germ-cells, verily, "this corruptible has put on incorruption and this mortal immortality"!

Now, no one to-day, qualified by his knowledge of embryology and genetics to the right of an opinion, would, I think, deny that the new organism is in the main the expression of what was in the germ-line, rather than of what it got directly from the body of its parents, but does this fact necessarily carry with it the implication that the germ is insusceptible to modification from without? Is not the serum of organisms with blood or lymph an excellent medium through which external influences may operate upon it? Is it not more reasonable to postulate the origination of germinal changes through some such mechanism as this than to attribute it to mysterious "spontaneous changes"?

With such thoughts in mind I and my research associate, Dr. E. A. Smith, set about making various tests.[2] Without attempting to tell you of our as yet unsuccessful attempts to secure cytolysins which will operate in the developmental stages of such periodically renewed structures as feathers, or to weary you with the history of our various other failures—of which there are an abundance—I wish to speak briefly about certain antenatal effects we secured in rabbits by means of fowl-serum sensitized against rabbit crystalline lens, and of the fact that such induced defects may become heritable.

[2] *Jour. Exp. Zool.*, XXXI, 2, Aug., 1920.

The crystalline lens of the rabbit was selected as antigen, and fowls as the source of the antibodies. The lenses of newly killed rabbits were pulped thoroughly in a mortar and diluted with normal saline solution. About four cubic centimeters of this emulsion was then injected intraperitoneally or intravenously into each of several fowls. Four or five weekly treatments with such lens-emulsions were given. Then a week or ten days after the last injection the blood-serum of one or more of the fowls was used for injection into pregnant rabbits. The rabbits had been so bred as to have the young advanced to about the tenth day of pregnancy, since from the tenth to the thirteenth day seems to be a particularly important period in the development of the lens. It is then growing rapidly and becomes surrounded by a rich vascular network that later disappears. From four to seven cubic centimeters of the sensitized fowl-serum were injected intravenously into the pregnant rabbits at intervals of two or three days for from ten days to two weeks. Several rabbits died from the treatment and many young were killed in utero. Of sixty-one surviving young from mothers thus treated, four had one or both eyes conspicuously defective and five others had eyes which were clearly abnormal. It is possible that still others were more or less affected, since we judged only by obvious, visible effects. We found later in some of the descendants of these individuals that rabbits which passed for normal during their earlier months subsequently manifested traces of defects in their lenses or in other parts of the eye.

The commonest abnormality seen in both the original subjects and in their descendants was partial or complete opacity of the lens, usually accompanied by reduction in size. Other defects were cleft iris, persistent hyaloid artery, bluish or silvery color instead of the characteristic red of the albino eye, microphthalmia and even almost complete disappearance of the eyeball. Taking into account the method of embryological devel-

opment, however—the relation of lens, optic cup and choroid fissure—the defects are probably all attributable to the early injury of the lens. In some cases, both among originals and descendants, an eye microphthalmic at birth may undergo further degeneration such as collapse of the ball and what appears to be a resorption as if some solvent were operating upon it. The eyes of the mothers apparently remained unaffected. This is probably due to the fact that the lens tissue of the adult rabbit is largely avascular and therefore did not come into contact with the injected antibodies.

That the changes in the eyes of the fetuses resulted from the action of lens antibodies is indicated by the fact that in not one of the forty-eight controls obtained from mothers which had been treated with unsensitized fowl-serum or with fowl-serum sensitized to rabbit tissue other than lens, was there evidence of eye-defects, and I may add, that among the hundred or more young obtained later from mothers which were being experimented upon with various types of sera or protein extracts, for other purposes, not a single case of eye-defect has appeared.

As already stated, once the anomaly is secured it may be transmitted to subsequent generations through breeding. So far we have succeeded in passing it to the eighth generation without any other than the original treatment. The imperfection, indeed, tends to become worse in succeeding generations and also to occur in a proportionately greater number of young. Though not analyzed completely as to its exact mode of inheritance, it has in general the characteristics of a Mendelian recessive. Like such anomalies as brachydactyly or polydactyly in man, the transmission is not infrequently of an irregular, unilateral type, sometimes only the right, at others only the left eye showing the defect. In the later generations, probably in some measure as the result of selective breeding, there is an increasing number of young which have both eyes affected.

To determine whether the reappearance of the defect was due merely to the passing on of antibodies or kindred substances from the blood stream of the mother, or to true inheritance, we mated defective-eyed males to normal females from strains of rabbits unrelated to our defective-eyed stock. The first generations produced in this way were invariably normal-eyed, but when females of this generation were mated to defective-eyed males again, we secured defective-eyed young after the manner of an extracted Mendelian recessive. It is obvious that in such cases the abnormality could only have been conveyed through the germ-cells of the male, and that it is, therefore, an example of true inheritance. Subsequent matings have shown that these young transmit the eye-anomalies as effectively as do individuals of the original lines. A new strain of defective-eyed young, established about the time our original paper went to press, is also flourishing and, as regards transmission of the defect, seems to differ in no way from the earlier stock.

But now, let us inquire as to where all this leads. Without entering into a discussion of just what, serologically, is taking place in the body or in the germ of fetuses borne by the lens-treated mothers, the point I wish to emphasize is that a certain specific effect *has* been produced; and, what is of greater moment, once the condition is established it may be not merely transmitted, but inherited. Whether the lens of the uterine young is first changed and then in turn induces a change in the lens-producing antecedents in the germ-cells of these young, or whether the specific antibody simultaneously affects the eyes and the germ-cells of the young is not clear. In any event it is evident that there is some constitutional identity between the substance of the mature organ in question and the material antecedents of such an organ as it exists in the germ.

Biologically considered, the most significant fact is that specific antibodies can induce specific modifications in the germ-cell. Whether these antibodies are trans-

mitted from the mother's blood or engendered in that of the young would seem to be of secondary importance. It stands to reason that antibodies originated in an animal's own body will modify germinal factors if corresponding antibodies introduced from without can accomplish this.

The whole question as to how important such a fact may be in contributing to an understanding of the causes of the germinal changes in organisms in general, which lead to variation and evolution, hinges on the question of whether changes in an animal's tissues will induce the formation of antibodies or kindred active substances in its own body. We have steadily accumulating evidence that such reactions do occur.

In our own laboratory, for example, after many attempts we have succeeded in securing a defective-eyed young rabbit from a mother of normal stock by injecting her repeatedly with pulped rabbit lens before and during pregnancy. Since the young rabbit in question has both eyes badly affected there can be no question that a rabbit can build antibodies against rabbit-tissue which are as effective as those engendered in a foreign species such as the fowl. We have likewise found it relatively easy to secure spermatoxins by directly injecting rabbits, both male and female, with rabbit spermatozoa. Moreover, a given male will develop antibodies against his own spermatozoa if he is injected intravenously with the latter.

We are also securing evidence that serologic reactions induced in the fetus through operations on the mother are not mere passive transmissions, but may become actively participated in by the tissues of the fetus. For example, female rabbits sensitized with typhoid vaccine followed by living typhoid germs may transmit to their young and even to their grand descendants the ability to agglutinate typhoid bacilli in serum diluted from 60 to 160 times. From the standpoint of heredity we have no reason so far for maintaining that this is anything but

placental transmission, though we are going to practice immunization generation after generation for a number of generations to determine if a truly hereditary immunity will be established. However, facts have come to light which show that there is more concerned in the operation than a mere transfer of antibodies from mother to fetus. For instance, the blood of young shortly after birth may show a higher titer than that of the mother. Again, after two or three months of development the young of certain of the sensitized mothers have shown a rather sudden rise in titer, much above that of the mothers. In such cases it would seem that some mechanism in the young rabbit itself is constructing antibodies which supplement those passively derived from the mother. Possibly in the process of development some organ important in such reactions just came into functioning. If this is true further experiments may throw some light on the perplexing question of the source or sources of the antibodies in an animal. After a few weeks, in such cases, the titer drops back again. In still another set of experiments we found that young from a sensitized mother, when nursed by a normal untreated mother, retained a fairly high titer for several months and even showed the rise of titer mentioned. On the other hand, young of an untreated mother when nursed by a sensitized mother acquired a fairly high titer from the milk of the foster mother but lost it rapidly after weaning time. Thus there are evidently constitutional factors operative in the young which have acquired their immunity through the placenta which are absent in the young whose antibodies were conveyed through food.

That changes in the blood serum may be caused by changed conditions in the tissues is further attested by many facts. For example, in pregnancy, the newly forming placenta may set free cells or cell-products which, sometimes at least, cause changes in the blood-serum of the mother, though the exact nature of these changes is

in dispute. Römer, using the complement-fixation technique, found that the serum of adult human beings may possess antibodies for their own lens proteins. Bradley and Sansum, employing anaphylactic reactions, found that guinea-pigs injected with guinea-pig tissue-proteins (liver, heart, muscle, testicle, kidney) develop immunity reactions. Again during the late war, the type of toxic action to which anaphylactic shock conforms was found to exist after extensive injury of the soft tissues. It resulted apparently from the absorption of poisonous substances of tissue origin into the circulation. In fact, various cells and tissues when injured liberate such poisons, and even blood in clotting is known to acquire a transient toxicity of this type.

With facts such as these before us, is it not a rational hypothesis to assume that changes in various parts of a body may on occasion influence the representatives of such parts in the germ-cells borne by that body? This appears all the more probable when we recall the facts learned from the study of precipitins and of anaphylaxis that each species of animal has a thread of fundamental similarity underlying the proteins of all its tissues. There is no reason to suppose that germinal tissue forms an exception. The further fact that homologous tissues, though existing in different species of animals, possess similar chemical characteristics, shows that to get an effect there need not be absolute identity between the protein with which the result is obtained and the original antigen. Since this is so, in order to have a lens antibody affect the germ, there need not be absolute chemical identity between the substance of such a tissue as the lens and the germinal constituents of which it is the expression. And if this is true for lens, why not for other tissues?

The blood-serum of any organism with blood thus affords a means of conveying the effects of changes in a parental organ to the germ-cell which contains the antecedent of such an organ. As long as there is little

change in the somatic element its germinal correlative would presumably remain constant, but any alterations of the soma which give rise to the formation of antibodies or other active agents, particularly if long continued, might induce changes in the germ. Such a hypothesis would seem to be plausible at least in accounting for degenerative changes such as the deterioration of eyes in such forms as the mole, or in fact, in the formation of vestigial organs in general.

On the other hand, there is no reason to infer that changes induced in the blood-serum may not also be instrumental in leading to progressive as well as regressive evolution. If we may have germinally destructive constituents engendered in the blood there is no valid reason for supposing that we may not also have constructive ones. When we learn more about what initiates and promotes growth in a part through exercise, or what causes hypertrophy of an organ, we may likewise find how corresponding germinal antecedents of that part may be enhanced. Until such time we shall probably remain in the dark regarding the mechanism of progressive germinal changes. As already indicated, in the hormones and chalones we have a wonderful series of secretions normally circulating in the blood and maintaining general physiological equilibrium. That reciprocal stimulations of various organs occur by this means is a well-established fact. Hypertrophy or atrophy of an endocrine gland may produce pronounced effects in the furthermost reaches of the body. Again we may inquire, is it reasonable to suppose that the germinal tissues will be inviolate to all this ebb and flow of chemical influence? Should we not expect specific reactions or selections here no less surely than in other tissues? Destruction of the pars buccalis of the hypophysis in the frog-tadpole will cause profound alteration in other endocrine organs such as the adrenals and thyroids, will retard the growth rate, render the entire organism albinous, and produce in the individual pigment cells a condition of sustained con-

traction. Shall we conclude that such a far-reaching influence as this, particularly in a developing organism, will pass the germ-cells by unscathed?

Similarly, growth in man is known to be controlled by a pituitary secretion that is carried by the blood to the various organs. The normal development of secondary sexual characters is determined by products from the testes or ovaries, and the activities of the generative organs themselves are intimately associated with the functioning of the adrenal and other glands. The periods of ovulation are inhibited by secretions from the corpus luteum; lactation is incited by products of the corpus luteum, the involuting uterus and the placenta; the carbohydrate metabolism in the liver and even in the most distant muscles is profoundly influenced by substances formed in the pancreas; the pancreas, liver, and intestinal glands are set to secreting through the stimulus of a product formed in the duodenal and jejunal mucosæ. And still others of such remarkable interrelations can be cited.

Truly one may pronounce that social complex of reciprocating individuals termed cells which make up an organism, "members one of another." And with all of these cooperative activities of the various parts of the body it is inconceivable, to me, at least, that the germ-cells, bathed in the same fluid, nourished with the same food, stand wholly apart.

May we not surmise then that as regards inheritance and evolution, Lamarck was not wholly in error when he stressed the importance of use and disuse of a part, or of modifications due to environmental change, in altering the course of the hereditary stream, particularly if we conceive of these influences as being prolonged, possibly over many generations? Have we not in the serological mechanism of the body of animals an adequate means for the incitement of the germinal changes which underly certain aspects of evolution?

DOMINANCE AND THE VIGOR OF FIRST GENERATION HYBRIDS

G. N. COLLINS

BUREAU OF PLANT INDUSTRY, U. S. DEPARTMENT OF AGRICULTURE

A STIMULATION of growth has come to be recognized as one of the results of hybridization. The phenomenon is of so much importance, practical as well as theoretical, that it has been given a special designation, heterosis. (Shull, 1914.)

New interest has been given to the study of the causes of this increased vigor by the work of Dr. Donald F. Jones[1] (1917 and 1918). Briefly stated the theory accepted by Jones is that growth is affected by a number of different characters or factors, the dominant members of each character pair being favorable and the recessive unfavorable to growth. Each strain or variety possesses some dominant and some recessive characters. When two strains are crossed the first generation of the hybrid exhibits the dominant characters of both parents and is in consequence more vigorous than either parent. In subsequent generations the number of dominant characters in any individual can not be greater than in the first generation and in a large majority of instances will be less, hence the average vigor of the second generation, although still above that of the parents, will be below that of the first.

The theory is not new, but has not been generally accepted because of outstanding objections. Dr. Jones has reviewed the earlier work and has advanced a very ingenious and entirely novel explanation of the objections. This explanation will be discussed later.

Bruce (1910) from purely mathematical considera-

[1] The theory has been further elucidated in the monograph on "Inbreeding and Outbreeding" by East and Jones (1920).

tions, showed that if dominance is correlated with vigor, crossing would produce "a mean vigor greater than the collective mean vigor of the breeds." Only a few days later appeared the paper of Keeble and Pellew (1910) with a concrete illustration and the suggestion that the "greater height and vigor which F_1 generations commonly exhibit may be due to the meeting in the zygote of dominant growth factors of more than one allelomorphic pair." It appears to me unfortunate that in elaborating this theory, Jones has retained the form of statement used by Keeble and Pellew, and describes the phenomenon of heterosis as due to the accumulation of dominant growth factors instead of placing the emphasis on the suppression of deleterious recessive characters. It may seem that the difference is only verbal since a dominant growth factor presupposes a recessive allelomorph. There is, however, a difference in the point of view, especially if the evolutionary significance of the phenomenon is considered. In speaking of dominant growth factors we seem to assume as a starting point, strains of low vigor subsequently improved by the appearance of dominant mutations. It is known that advantageous variations, whether dominant or recessive, are of extremely rare occurrence and while evolutionary progress as a whole must be dependent on such rare progressive changes the effect of these is negligible as a factor explaining heterosis.

Heterosis in Maize

In all varieties of maize there are to be found plants that are abnormal in some particular and these abnormal individuals are almost always deficient in vigor and yield. When varieties are self-pollinated for a series of years at least a large part of the degeneration that follows is caused by these abnormalities.

The bearing of abnormalities on heterosis will be more easily understood if the behavior of two or three examples is described.

A very common abnormality consists of small yellow spots thickly distributed on all the leaves which develop later than the seedling stage. While undoubtedly interfering with the proper functioning of the chlorophyl, the effect of this abnormality is not serious. Even in breeding experiments seed may be saved from a spotted plant and in a population containing these partly chlorotic individuals many of the ovules on the most vigorous plants will be fertilized by pollen from affected plants. It is easy to see how characters of this kind persist.

A more serious and less common abnormality is one that prevents the leaves from unrolling properly, with the result that the plant is bent and contorted and in extreme cases never reaches maturity. Seed would seldom be saved from plants affected with this disorder, but they frequently produce pollen in normal quantities and the character in consequence is widespread and difficult to eliminate.

Albino seedlings may be taken as an example of a still more serious type of abnormality. In this case all individuals that show the character die in the seedling stage. It might appear at first that disorders of this type would be self-eliminating. The character is recessive, however, and in many strains there are plants which are hybrid for the albino character. These hybrid plants show no trace of the character, yet one half of the pollen grains and one half of the ovules carry albinism. If either of these unite with one of their kind an albino plant results, while if they unite with a normal gamete another hybrid plant like the parent is produced. Such characters may be carried along in this manner for any number of generations in a completely latent form, coming into expression only when pollen grains and ovules both bearing the character chance to unite.

Breeding experiments have shown that the more conspicuous of these abnormalities are recessive Mendelian characters which have come into expression through the chance meeting of male and female gametes both bearing

the character. Only a few of the more obvious of these abnormalities have been studied, but there is no line of demarcation between these conspicuous changes and those that are less evident down to variations that can not be distinguished visually from environmentally induced fluctuations.

Different varieties possess different assortments of deleterious characters and in a cross between two unrelated strains all of the recessive lethal or semi-lethal characters[2] not common to both parents are kept from expression, since the recessive characters of each parent are brought into combination with, and suppressed by, their dominant allelomorphs in the other parent. Freed from the depressing effects of these recessive characters, the first generation of a hybrid is usually more vigorous than either parent. In subsequent generations the old recessive characters again come into expression in some of the plants, thus reducing the general vigor below that of the first generation.

If the above explanation of heterosis is to be accepted it should follow that a majority of the departures from the normal must be deleterious and recessive while those which are advantageous are dominant.

The existence, on the other hand, of advantageous recessive, or deleterious dominant, variations would operate to make F_1 populations less vigorous than the average of their parents and conversely inbreeding would tend to increase vigor.

Variations in Maize Chiefly Deleterious and Recessive

None of the recorded Mendelian variations of maize is of a nature that would be advantageous to a wild

[2] The term character has been used in many places in this paper where it would have been more in conformity with current usage to employ the term factor.

Since heterosis results from the combined action of independent units it might seem proper to call these units factors. Taken individually, hovewer, each of the units is assumed to produce a tangible effect and is, properly speaking, a character.

plant and most of them are obviously detrimental. Moreover, if variations occur at random the chances are almost infinitesimal that any particular variation would constitute a favorable addition to the complex mechanism of a highly specialized plant or animal. A chance alteration in the parts of a machine would seldom improve its efficiency.

Of the recorded heritable variations in maize the departure from the normal condition is recessive in a great majority of the cases. Aside from a number of widespread characters where neither member of the allelomorphic pair may be considered more normal than its mate, the only dominant variations in maize that come to mind are pod corn and fasciated or bear's foot ears.

On the other hand, the recessive variations already described number more than 20 and it would be safe to say that hundreds of others are known to maize breeders.

In a complex organism we may expect that deleterious variations will occur more frequently than beneficial variations, but that such a large proportion of the characters should be recessive calls for comment. East and Jones hazard the suggestion that natural selection has suppressed the tendency to produce dominant unfavorable variations while the tendency to produce unfavorable recessive variations has been tolerated.

It should be kept in mind that the observed preponderance of recessive characters does not necessarily imply that a corresponding preponderance of mutations or germinal changes are recessive. Dominant disadvantageous variations are eliminated much more promptly than recessive and the gradual accumulation of recessive characters soon would place them in the majority in any cross-bred species. It seems not improbable that the great preponderance of recessive over dominant characters is a measure of the extent to which dominant characters are eliminated. In a cross-bred form even variations that result in sterility or death may persist indefinitely if recessive. It may well be that the rate at which

dominant characters appear represents roughly one half of the germinal changes that are taking place, new recessive characters originating at approximately the same rate. The preponderance of recessive characters would then be explained, as the result of their preservation in a hybrid condition.

Minor Variations Occur with Greater Frequency than Major Variations

This assumption is made necessary by the fact that the abnormalities which are sufficiently conspicuous to be identified and isolated will account for a part only of the reduction of vigor that follows inbreeding. A part must be due to the combined effect of minor unfavorable variations, the effect of individual variations being insufficient to produce changes that can be distinguished from environmental fluctuations.

That minor variations are more numerous than major is almost self-evident if large and small variations form a continuous series, as they seem to do, since there is a limit to the largeness of variations but none to their smallness. If further proof is needed it follows from the fact that most major variations can be resolved into less comprehensive variations and these subsidiary or minor variations must be more numerous than the major variations of which they form parts.

As East has noted, our classification of variations into large and small may have only a remote relation to the importance of characters in the plant's economy. But whether judged by the change in appearance or by their importance to the organism, it is certain that larger or more fundamental changes must occur less frequently than smaller or less important variations.

The Nature of Variations in Maize

The appearance of deleterious characters when maize is inbred and their disappearance when crosses are made, would follow whether the characters were the result of recombination or of mutation.

There seems to be no sure way of distinguishing between the behavior of characters that appear as the result of recombination and those that result directly from a germinal change. Changes that occur in homozygous strains must be mutations. It is, however, theoretically impossible to be certain that a strain is homozygous regardless of the number of generations that it has been selfed and, practically, the criterion of homozygosity is fixed by the accuracy with which comparisons can be made. With quantitative characters in maize it is difficult to detect with certainty differences of less than 10 per cent., yet sister progenies of strains that have been selfed for as many as 8 or 9 generations usually show differences too large to be ascribed to chance. This difficulty of obtaining uniformity may be due to the large number of factors involved, but also may be due to the frequency of minor mutations. If a new character appears in a relatively uniform strain that has been selfed for a number of generations and the character behaves as a simple Mendelian unit, it usually is ascribed to a mutation. Even in such cases, however, the character may be due to recombination. If the two factors of a dihybrid recessive character arose independently in nearly the same position on homologous chromosomes, the close linkage of the dominant allelomorph of one factor with the recessive allelomorph of the other would long postpone the appearance of an individual with both recessive factors and when it did appear the departure of its behavior from that of a simple character would be difficult to detect.

Although the nature of variations does not affect the bearing which the preponderance of recessive characters has on the explanation of heterosis, there are practical as well as theoretical reasons for wishing to know whether new characters that appear in breeding stocks are mutations or result from the combination of factors already present in the germ plasm. If the undesirable characters that appear from time to time, even in well-

bred varieties, are the result of recombination, the breeder will be encouraged to expend the time and labor necessary to eliminate them. If, on the other hand, these new characters are the result of an unstable germ plasm other means must be sought.

Already the importance of deleterious recessive variations has found application in the breeding of maize. It soon was realized that to successfully eliminate recessive characters it is necessary to bring the characters into expression by inbreeding. Once a strain has been freed of undesirable characters, vigor may be restored by combining the inbred lines or the full advantage of dominance may be realized by growing first-generation hybrids of the better strains.

This method of breeding will be relatively unsuccessful if unfavorable mutations are of frequent occurrence. It is perhaps too early to be sure that this is not the case, but it is encouraging that in strains self-pollinated for 13 generations Jones finds no conspicuous variations after about the 7th generation. The next step is to demonstrate that no unfavorable variations appear when the selfed lines are crossed. This has been shown to be the case in the first-generation, but a certain percentage of multiple factor recessives are to be expected in subsequent generations.

If linkage is operative these recessive characters would come to light slowly, much as they appear in successive generations of an open-bred variety. As already pointed out, there is as yet no way of distinguishing between mutations and multiple factor characters, when the factors are linked.

Nature of Degeneration That Follows Inbreeding.

In discussing the nature and causes of the reduction of vigor that follows inbreeding, it is necessary to choose words with great care. To state the questions at issue in such a way as to distinguish between differences of fact and differences in the use and meaning of words will tax the possibilities of the language.

Many of the older writers on heredity have held that inbreeding is a cause of degeneration. In avoiding ambiguous words "cause" is one of the first that must go. If forced to define their position this school would probably be content with the statement that degeneration is a necessary consequence of inbreeding, the intermediate steps or nature of the process being unknown. Is this conception really at variance with the idea that degeneration results from the increased number of unfavorable recessive characters brought into expression by increasing homozygosity? Does not this conception rather amplify the older, general and indefinite position by explaining how the degeneration may be brought about?

It excites unnecessary opposition, and is not entirely fair, to read into the early writings the idea that inbreeding was held to be the immediate and direct cause of the subsequent degeneration. Such words as "cause" and "per se" have perhaps been used, but is there not sufficient latitude in their meaning to allow the later discoveries to be looked upon as explaining rather than refuting the old doctrine?

In the attempt to bring the two views into sharp contrast the newer explanation is sometimes stated in terms which likewise must be interpreted with latitude if the explanation is to be accepted. Thus East and Jones (p. 123) state one of the results of inbreeding maize as follows: "There is a reduction in size of plant and productiveness which continues only to a certain point and is in no sense an actual degeneration." It is difficult to imagine a degeneration more "actual" than that usually following the inbreeding of maize.

In another place (p. 139) the same authors say: "The only injury proceeding from inbreeding comes from the inheritance received." Such statements have an unfortunate air of finality that probably was not intended. The relation between inbreeding and degeneration has been greatly clarified by the work of these authors, but the above statement taken literally places them in a posi-

tion similar to that of the older writers who stated that inbreeding was the "cause" of degeneration. There may well be other and important ways in which inbreeding is associated with degeneration.

There is for example definite evidence that vigor is reduced by continued asexual reproduction (Shull, 1912), and although it may be urged that this result is associated with the phenomenon of senescence, so may be the decline of vigor that follows inbreeding.

Furthermore, it has been shown by Calkins (1919) that in the ciliate, *Ursoleptus*, conjugation between sister cells of an asexually propagated line increases vigor.

Objections to the Explanation

Two objections have stood in the way of accepting dominance as an explanation of heterosis. The first of these is that if this explanation is the correct one it should be possible to obtain an occasional F_2 individual, homozygous for all dominant allelomorphs, the progeny of which should be uniformly as vigorous as the F_1. It is held that no such F_2 individual has been found. The second objection is that the distribution in F_2 should be skew with the mode above the mean while in fact F_2 populations show a symmetrical distribution.

Jones has proposed a novel and ingenious explanation of both objections. He has pointed out that it is only necessary to assume that the phenomenon of linkage, which plays such an important rôle in the inheritance of *Drosophila*, is operative also in maize.

If groups of characters are inherited as units with little or no crossing over, both dominant and recessive characters being represented in any particular unit, the first generation would still exhibit all the dominant characters of both parents, but when segregated into pure lines each pure line would again exhibit recessive characters, with a consequent decline in vigor.

This assumption of group inheritance or linkage would also meet the objection that F_2 populations exhibit a normal and not a skew distribution.

There are a number of instances of coherence or linkage known in maize, but if the characters studied to date are a fair sample the rôle of linkage must be of minor importance. Linkage relations have not been studied in sufficient detail even to state with assurance that the characters are arranged in linear series corresponding to the number of chromosomes, although this conclusion is indicated. The linkages of most of the Mendelian characters are very loose and it would seem necessary to conclude that, if the characters of maize are arranged in a linear series, the chromosomes must be either very long or very flexible.

While admitting that linkage would meet the objections urged against the simple hypothesis that the suppression of recessive characters explains heterosis, it may be well first to make sure that any such assumption is necessary. An examination of the maize literature indicates that the difficulty of securing uniform strains with the vigor of the first generation has been assumed rather than demonstrated. No case was found where selection following hybridization had been continued long enough to approximate homozygosity. There are also very few cases where the more vigorous F_2 individuals have been chosen as parents of the F_3. The most extensive series of experiments are those of Emerson and East (1913).

Height is probably the most satisfactory character to use as a measure of heterosis. There are 23 comparisons of F_1 and F_2 populations in the work of Emerson and East. To these six can be added from our own experiments.

In these 29 cases the mean of the F_2 was below that of the F_1 in every instance but in ten of the 29 cases the largest of the F_2 plants equalled or exceeded the largest of the F_1 individuals and in every case where a progeny was grown from a plant near the upper limit of the range of the F_2 its mean exceeded that of the F_1.

Other characters reported by Emerson and East for

which the F_1 was measurably larger than the mid-parental value are length and diameter of ear and length of internode. With respect to length of ear (Tables XIII–XV), there are 13 F_2 progenies that may be compared with the F_1. In 10 the mean was higher than the mean of F_1. Four F_3 progenies were grown from F_2 individuals above the mean of F_1 and in 3 of these the mean exceeded the mean of F_1.

With respect to diameter of ear (Tables XVIII and XIX), 8 F_2 progenies may be compared with the F_1. In 2 of the 8 instances it would appear that the mean was above that of F_1. Seven of the 18 F_3 progenies were grown from F_2 individuals above the mean of F_1 and in every case the F_3 mean exceeded the mean of F_1.

Length of internode is the character showing the most decided increase in F_1 over the mid-parental value. In the two crosses reported (Tables XXXIII and XXXIV) this increase was 33 and 27 per cent. None of the F_2 progenies grown the same season as the F_1 equalled the F_1. The mean of the F_1, however, was exceeded by the mean of 5 of the F_3 progenies grown the following season, although the parents were not selected for internode length. The results with these characters give little or no evidence of non-heritable vigor in the F_1, neither is there any proof that it is difficult to select progenies with the vigor of F_1.

Expectation of Obtaining F_2 Progenies with the Vigor of F_1

In the absence of experimental data, to secure which will require very extensive experiments extending over many years, it may be instructive to consider what results may be expected if suppression of semi-lethal characters is the true explanation of heterosis.

The difficulty of obtaining individuals homozygous for all or even a limited number of characters has been frequently pointed out, but the bearing of this on the difficulty of retaining the vigor of the first generation seems not to have been appreciated.

With a sufficiently large number of characters influencing vigor it would be impossible in practice, even without the assumption of linkage, to obtain homozygous individuals having the vigor of the first generation.

Thus with 10 pairs of characters over 700,000 individuals would have to be grown before there would be an even chance of obtaining an individual homozygous for all of them.

More than ten separately inherited Mendelian character differences affecting growth have been identified and there is no reason for believing that more than a small proportion have been isolated or that more than a small proportion produce conspicuous morphological changes that would be readily detected.

A near approach to the vigor of the F_1 might be expected, of course, without complete homozygosity.

Some idea of the chances of isolating strains that are practically homozygous may be obtained by calculating the size of the populations that must be grown to insure a reasonable chance of finding an individual homozygous for say 70 per cent. of the characters.

Table I indicates the size of the populations necessary to fulfil these conditions with the number of character pairs ranging from 10 to 30.

TABLE I

Number of Pairs of Characters Involved	Number of F^2 Individuals Necessary to Provide an Even Chance of Obtaining at Least One Individual Homozygous for 70–90 Per Cent. of the Character Pairs		
	70 Per Cent.	80 Per Cent.	90 Per Cent.
10	199	1,760	23,400
15	6,000	56,000	16,150,000
20	23,600	1,800,000	429,000,000
25	457,000	39,100,000	28,200,000,000
30	2,470,000	1,710,000,000	7,070,000,000,000

It will be seen that to have a reasonable chance of obtaining an individual homozygous for even 70 per cent. of the character pairs it is necessary to limit the character pairs to 15 or less. Another way of gaining a quantitative idea of the degree of homozygosity that

TABLE II

NUMBER OF F_2 INDIVIDUALS NECESSARY TO PROVIDE AN EVEN CHANCE OF OBTAINING AT LEAST ONE INDIVIDUAL HOMOZYGOUS FOR THE NUMBER OF DOMINANT CHARACTERS INDICATED IN COLUMN ONE.[1]

Number of Dominant Homozygous Characters	Number of Characters Involved													
	2	3	4	5	6	7	8	9	10	11	12	13	14	15
2	11	5	3	2	2	1	1	1	1	1	1	1	1	1
3		45	14	7	4	3	2	2	2	2	2	2	1	1
4			178	45	19	10	6	4	3	3	2	2	2	2
5				710	150	54	26	14	9	6	5	4	3	3
6					2840	516	165	70	35	20	13	9	7	5
7						11400	1820	516	199	94	49	29	19	13
8							44900	6520	1760	584	246	123	68	41
9								182000	23400	5500	1770	700	322	165
10									726000	85400	18400	5490	2030	870
11										2900000	313000	62600	17400	6000
12											11600000	1160000	215000	56000
13												46500000	4320000	750000
14													186000000	16150000
15														744000000

[1] The determinations are carried to only three significant figures.

may be expected is presented in Table II, which gives the number of individuals necessary to provide an even chance of obtaining at least one individual homozygous for different numbers of characters in crosses when from two to 15 character pairs are involved. It should be kept in mind that even though one should obtain an individual homozygous for a sufficiently large percentage of the characters involved to approximate closely the F_1 in vigor, it would be necessary to grow a progeny from this individual before its inherent vigor could be demonstrated. The numbers given in the tables might thus be taken to represent F_3 progeny rows instead of F_2 individuals.

The conclusion is that perjugate progenies equalling or even closely approximating F_1 in vigor are hardly to be expected in breeding experiments and consequently no assumptions are necessary to account for their non-appearance.

SKEW DISTRIBUTION DUE TO DOMINANCE

The second objection, that of the failure of F_2 progenies to show a skew distribution, may now be considered. There can be no question that a series of independent, dominant characters influencing size would bring about a skew distribution. Assuming the characters to have equal effect, two characters would give a distribution of 1, 6, 9, three, a distribution of 1, 9, 27, 27, and with four characters the distribution would be 1, 12, 54, 108, 81. It is apparent that with an increase in the number of characters the skewness becomes less pronounced. It may be of interest to determine whether, with a reasonably large number of characters, the skewness would be detected in populations of the size usually grown in experiments.

With 20 pairs of characters giving 21 classes 1,099,-514,627,776 individuals would have to be grown to obtain a representative population. Of this population 99.91 per cent. would fall in the 12 classes with the largest

number of dominant characters. That is, populations of over 700 would have to be grown before there would be an even chance of getting any individuals smaller than those represented in these 12 classes. With ordinary sized populations then the distribution would be fairly represented by the distribution of the 12 largest classes.

The distribution among these 12 classes would be as follows:

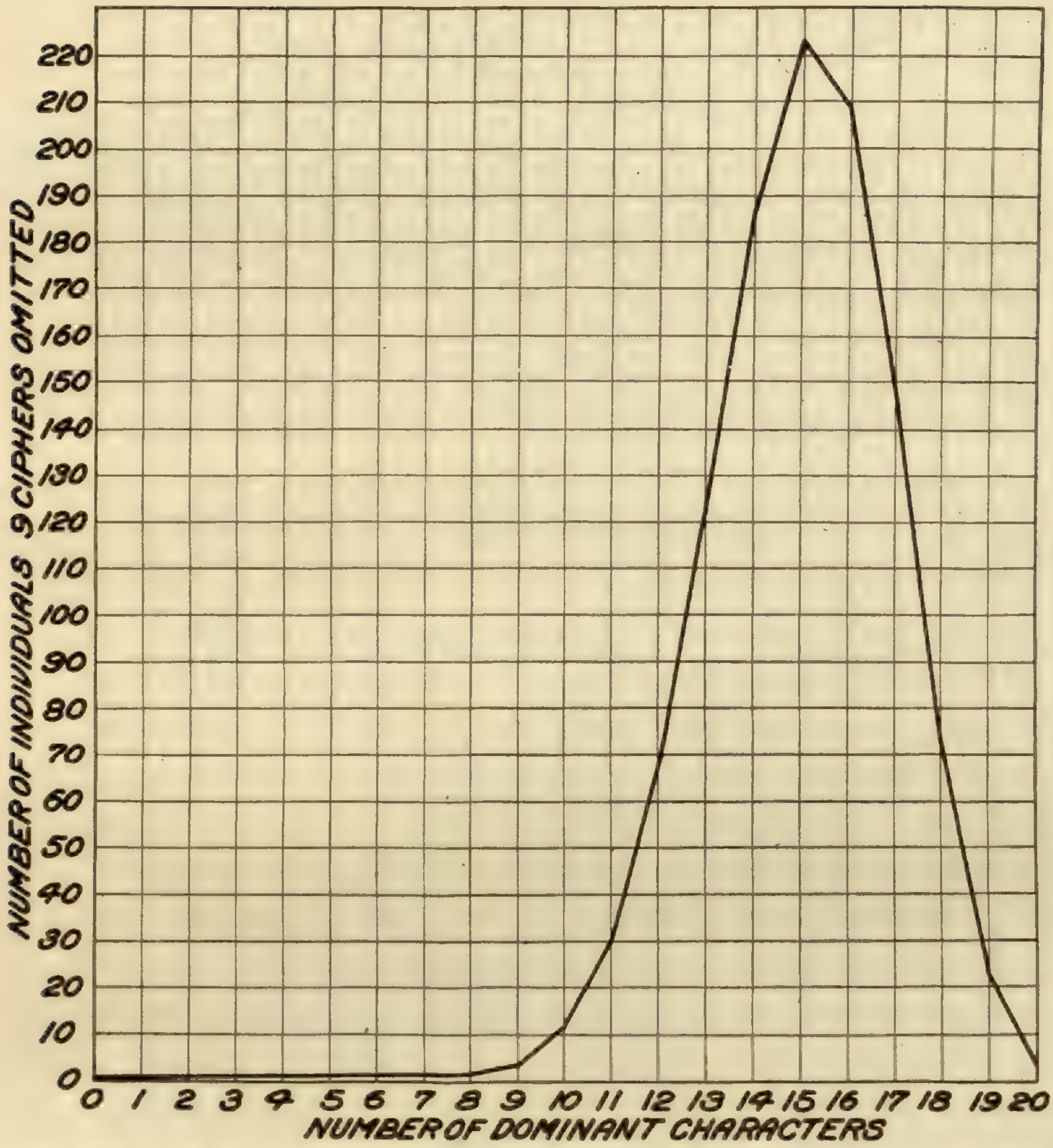
No. Dominant Allelomorphs	Proportion of Individuals Expected
9	.3
10	1.0
11	2.7
12	6.1
13	11.2
14	16.9
15	20.2
16	19.0
17	13.4
18	6.7
19	2.1
20	.3

A distribution of this nature, with populations of approximately 500 individuals, conforms to the normal frequency curve as closely as would be expected. The mode departing from the mean by only 3/100 of a class.

The theoretical distribution of an F_2 population involving 20 pairs of characters of equal weight with complete dominance is shown in the accompanying diagram. It will be noted that although the curve as a whole is skew, the portion to the right of the class with 9 dominant characters, which comprises 99.91 per cent. of the area, is practically symmetrical.

With 10 character pairs there would be 11 classes and 99.65 per cent. would fall in the 7 largest classes and in this portion of the theoretical population the mode would be separated from the mean by only 3/10 of a grade.

Even should it be possible to grow F_2 populations sufficiently large to detect departures from a normal frequency distribution there is yet another reason for questioning that a skew distribution should be expected, when



Distribution of individuals in an F_2 population involving 20 pairs of characters of equal weight and showing complete dominance.

plotted in the customary way. It has come to be accepted that the effect produced by a given growth factor is dependent on the size of the organism. For example, if a growth factor increases the length of the internode by a given amount, it is clear that the height of a plant with 30 internodes will be increased more than that of a plant with only 15 internodes. In other words, the effects are factorial instead of additive. A convenient method of classifying a population on a factorial basis has been proposed by Zeleny (1920), who takes the range of each class as a constant percentage of the value of the mid

point of the class. The result of this change in plotting is to increase the range of sizes included in the higher classes and consequently to raise the mode.

In conclusion it would seem, therefore, that the assumption of linkage, while perhaps not improbable, is superfluous so far as the explanation of heterosis is concerned, since neither of the objections which it was framed to meet have foundation in fact.

It is, perhaps, too much to assert that the suppression of deleterious recessive characters completely explains heterosis or that the reappearance of these characters is the only factor in the decline in vigor that follows inbreeding, but the behavior of maize is in full accord with this explanation.

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CORRELATION OF TAXONOMIC AFFINITIES WITH FOOD HABITS IN HYMENOPTERA, WITH SPECIAL REFERENCE TO PARASITISM[1]

PROFESSOR CHARLES T. BRUES

Bussey Institution, Harvard University

Entomologists can all agree that the attachment of most phytophagous species belonging to the more highly specialized orders of insects is very firmly fixed, and the Hymenoptera form no exception. We can also agree, although in less definite terms, that many parallels exist among plant-eating Hymenoptera between taxonomy and food habits. I do not propose to treat of this series, however, partly because I am not sufficiently familiar with them, but also on account of the great interest which attaches to the parasitic groups of Hymenoptera.

During the last decade our conception of the process of nutrition in insects has undergone considerable change, due to the discovery that various microorganisms form an important part of the food supply of many forms. It is quite certain that certain saprophagous, sarcophagous and coprophagous ones probably feed directly, not at all upon decaying and fermenting plant materials, carrion or excrement, but upon the bacteria, yeasts, etc., always abundant in organic material undergoing decomposition. We must judge of the protein requirements of such insects not by the gross substances or substratum, but on the basis of the microorganisms present (Baumberger, '19).

This aspect does not appear to enter into the economy of the Hymenoptera, although there may be a relation between fungi and nutrition in some Cynipidæ, as the

[1] Contribution from the Entomological Laboratory of the Bussey Institution, Harvard University, No. 181.

galls of these insects are commonly invaded by fungi and yeast-like organisms. On the other hand, these same gall-wasps exhibit remarkable correlations between structure and habits as shown by many workers, most elaborately and clearly by the recent studies of Kinsey ('20). The Cynipidæ are at present restricted to a very limited series of plants on which they induce the formation of galls. With the exception of some undoubtedly primitive forms, they occur almost exclusively on Rosaceæ of the genera *Rosa* and *Rubus*, and on the unrelated genus *Quercus*, the latter harboring a very large number and a far greater variety of forms. In the gall wasps, we see, therefore, a nearly exclusive association with a very few genera of plants and what is still more striking is the fact that the more primitive ones, although few in number, exhibit a wide range of food-plants. This leads to the inevitable conclusion that we can trace the evolution of host relations in this group as now living, from a very generalized condition to a highly specialized one.

Many other insects, particularly Homoptera, harbor certain probably symbiotic organisms, and recent studies (Brues and Glaser, '21) tend to show that these may be very important factors in the nutrition of the insects. Almost nothing is known concerning such organisms in Hymenoptera, but if they are present in some cases, as seems probable, they must be reckoned with in any complete studies of food-habits.

The relation of food-habits to taxonomy in the Hymenoptera becomes particularly interesting in connection with the appearance of parasitism in several forms, which is of widespread occurrence in the order. In fact, a large proportion of the Hymenoptera are parasitic, and with the development of this mode of existence have come such elaborate structural modifications, and specialization in behavior, that species have multiplied at a very rapid rate. From this apparent chaos, even taxonomists have as yet brought only very partial order, and any discussion of parasitism in the Hymenoptera is

necessarily very incomplete and may be misleading in some details.

In the Hymenoptera the designation "parasitic" has been applied to habits of extremely diverse nature, and this very loose use of the term may easily lead to serious confusion unless we consider the matter carefully before proceeding further. It is most commonly applied to several large and abundant groups whose members live in the larval condition in other insects which they almost invariably destroy after attaining full growth. Such habits are quite similar to those encountered in other orders of insects, although in no other do they attain such a high degree of specialization; nor do they involve such a series of unrelated smaller groups, with the possible exception of the Diptera. While this is the most abundant and widespread type of parasitism among insects, we must not lose sight of the fact that it is a rather unusual condition so far as animals and plants in general are concerned, in that the parasite and host belong to the same class and are thus closely related. A similar relation exists among the Crustacea where certain decapods are parasitized by other members of the same class, and even among Protozoa there are ciliates parasitic in the bodies of other infusorians. Such associations are very rare, however, and the vast majority of parasitic Crustacea and Protozoa, as well as other parasitic animals, depend upon animals far removed from themselves for hosts, although there is very generally a close correlation between the host and parasite in that related parasites depend upon related hosts. Another peculiarity of this type of insect parasitism lies in the prompt death of the host, which does not usually ensue as a result of other animal parasites, although it is a common result of presence of some protozoan parasites of the higher animals. In others, again, like the well-known nematode, Trichina, the ultimate death of the host is necessary for the continued propagation of the parasite, but actual death normally results from other causes. Still another charac-

teristic of this type of insect parasitism is its restriction to the larval stages, although extending over the entire growth period. This is by no means unique among animals, but it is one of the distinguishing characteristics between this type of parasitism and the one next to be considered. In its perfected state this relation between host and parasite is a marvelously balanced association and one which we might expect to furnish valuable data on the correlation between taxonomy and habits.

A second type of parasitism encountered in the Hymenoptera is that exemplified by most of the parasitic bees and wasps. This has recently been discussed by Wheeler ('19). Here the parasitic larva is really at first predatory so far as food-habits are concerned, devouring the host larva shortly after hatching. The sequence of events is initiated by the preparation of the larval food-supply of the host by the mother bee or wasp. Most solitary wasps store up, in a nest which they have prepared, one or more insects which they have paralyzed by means of the sting, and attach one of their eggs to the body of the prey. Under normal conditions the larva hatching from this egg consumes the prey, attaining full growth, and later completing its metamorphosis. Bees behave in much the same manner, but the store of food in the nest consists of honey and pollen. When parasitism intervenes, the egg of the parasite is also placed upon the food supply, and on hatching, the larva of the host and parasite find themselves in proximity, each ready to appropriate the contents of the nest. In numerous cases that have been studied (Graenicher, '05), the larva of the parasitic form has more powerful jaws than its rival, and encounters little difficulty in destroying it. It now proceeds to consume the food-supply exactly as the host larva would have done, casting off its enlarged mandibles at the first molt. Thus the actions of the larva savor not at all of parasitism, but it is in the fixed habits and instincts of the adult, which require the nests of particular wasps and bees, that the parasitic relation holds.

Correlated with such habits, structural modifications of the body appear, such as the loss of the pollen-collecting apparatus in parasitic bees.

In certain ants there occurs a third type, social parasitism (Wheeler, '04) whereby the young females of some ants that do not establish their own colonies insinuate themselves into the nests of other species of ants, do away with the queen, and take on themselves the function of egg-laying. As the larvæ from these eggs are raised to maturity, they produce worker individuals of the parasitic species which gradually supplant the original population. Finally, the colony becomes pure and maintains itself through its own efforts, giving no evidence of the temporary social parasitism by which it has originated. In a very few cases social parasitism may become permanent with the complete elimination of the worker caste.

The term entomophagous parasite may be applied with some appropriateness to all of the three types described, but is most suitable for the first one, since there the parasite not only consumes its host, but feeds upon nothing else during its developmental stages. By far the largest number of species in the order exhibit this type and it is the only one which I shall consider in any detail.

There are several ways in which such parasitism may have originated, but the question of origin is best deferred until its several phases have been discussed at greater length.

Defining parasitism in its several forms as enumerated on a previous page, we find that there are parasitic genera included in nearly all of the larger groups of Hymenoptera. Thus, the Ichneumonoidæ, Serphoidea and Chalcidoidea, each represented by a number of families, are almost exclusively entomophagous parasites, while in the Aculeata, numerous parasitic genera appear, scattered through a series of families with generally non-parasitic habits. In addition to these there is the primitive family Oryssidæ, now known definitely (Rohwer,

'17) to be parasitic, and a few other families nearly all of somewhat doubtful affinities. Thus of the nearly one hundred families included in the order, between forty and fifty are composed either entirely or almost exclusively of genera with parasitic habits, the remainder being phytophagous or predatory with isolated cases of parasitism, among both the predatory series, and one of the phytophagous ones.

Considering these larger groups, the suborders and superfamilies, more in detail we find that the most primitive of all known Hymenoptera, the suborder Chalastogastra, are phytophagous. Of these, about a dozen families, comprising the sawflies or superfamily Tenthredinoidea, are almost exclusively defoliating forms, feeding in their larval stages on the leaves of various flowering plants. Another family, the Siricidæ, feeds internally on the tissues of woody plants, and, at least so far as food-habits are concerned, there are two other families which form a transition between the sawflies and wood-wasps. It is in the groups above these that the parasitic habit appears, and with the possible exception of one family, the Oryssidæ, to be mentioned later, all these groups are usually associated as a second suborder, Clistogastra, contrasted to the more primitive Chalastogastra. Among them several groups of families, conveniently classed as superfamilies, are three extensive parasitic ones: first, the Ichneumonoidea, comparatively large species comprising about half a dozen families; second, the Chalcidoidea, represented by small or minute species comprising fully a dozen families; third, the Serphoidea another half dozen, mainly very small species. Together with a part of the Cynipoidea, these form the enormous complex commonly known as the Hymenoptera Parasitica. All are quite closely related, but rather easily grouped and distinguished, in spite of certain annectant and aberrant families.

The habits of the several series are also very uniform.

The egg is nearly always laid upon the body of the host or thrust into it, usually the latter, to which purpose the extrusible, stiletto-like ovipositor of the female is adapted with great nicety. Oviposition may take place either in the egg of the host, in the larva, or even in a later stage, and the parasite may complete its development either in the stage of the host in which it is laid, or development may be delayed and not completed till the host has proceeded to a further stage in its ontogeny. Under such conditions the larva is to a great extent passive, although in its earlier minute stages it frequently exhibits (*e.g.*, in certain Serphoidea) great modifications in body form, and develops monstrously specialized jaws or other organs to aid in attacking the massive tissues or yolk-masses of its host.

When such modifications of the young larva are transitory and disappear almost completely after one or two ecdyses, they form a transition to several very clearly defined cases of hypermetamorphosis which have been noticed in certain Chalcidoidea by several observers (Wheeler, '07; Smith, '12, and Brues, '19). In members of two families, the Eucharidæ and Perilampidæ, they have found an active, free-living, first stage larva known as a planidium which is quite similar to the triungulin of the Meloid beetles and the Strepsiptera. Like them, the planidium becomes helpless once it has become parasitic. Great interest attaches to the planidium, but until its distribution is much better known it can not be considered of taxonomic value, especially as quite similar larvæ are known in several other orders of insects. Another series of Hymenoptera, certain parasitic bees, are known through the researches of Graenicher ('05) and others to possess much larger jaws in the first larval stage. As we have mentioned previously, the type of parasitism in this case is very different, for the parasite simply eats the host larva that it may appropriate its food-supply, and we have a parallelism in structure, of

independent origin, and hence of no classificatory importance.

Comparative anatomy and post-embryonic development show very clearly that, with the exception of some secondarily phytophagous forms, only the primitive Hymenoptera are phytophagous. As one can not seriously question the monophyletic origin of the order, the varied food-habits now represented must have been derived from some form of vegetarianism.

In all of the higher Hymenoptera or Clistogastra, active and aggressive characteristics are very prominent in the behavior of the adult females, whatever may be the food-habits of the larvæ. Thus in the wasps, the parent captures as prey suitable insects with which to feed her larvæ, or to provision her nest, if her young are to receive no post-natal care. In all cases she prepares some sort of a cell or nest for her brood, and frequently this requires marvelous skill in the selection of particular materials and the collection of specific insects for food. Where nests are provisioned in advance, the prey is stung and paralyzed after a manner that requires very complex instinctive behavior. If, on the other hand, we look at the activities of the larva of one of the wasps that stores a paralyzed insect away and places her egg upon it, we see the larva consuming its food supply much after the fashion of an externally feeding entomophagous parasite. In fact, it is difficult to distinguish any really fundamental differences. In each case the host is stung and the egg attached to it, always externally by the wasp, but sometimes externally also by the parasite. The wasp paralyzes her prey, which the parasite does not do, as her sting is not so severe, and she does not further bother with the host insect. The egg of the parasite is deposited at the time of stinging, and that of the wasp by a later operation of the same organ, the ovipositor with which she has previously paralyzed, but not killed, her prey. Thus, aside from the maternal instincts, the entomophilous wasp is scarcely more different from the ichneumon-

fly, than some ichneumons from others.[2] Equally varied habits exist in at least a few cases even in a single species of ichneumon, for certain Itoplectis may be either parasites of caterpillars, hyperparasites, or inhabitants of the egg-cocoons of spiders where they devour the contained eggs. From the entomophilous wasp has been developed the parasitic one and we have alluded to its origin as traced by Wheeler ('19).

From the foregoing, it is seen that we might derive the habits of the wasp from those of the parasite, or *vice versa*, with but little difficulty, although the more elaborate instincts of the wasp appear more naturally as the latter development.

If now we return to the free-living phytophagous Chalastogastra, it appears for morphological reasons especially that the entomophagous ichneumon flies have been derived directly from them and I think that the transition from phytophagy to parasitism is quite clear. Whether it involves the interpolation of predatism or sarcophagy is perhaps more a matter of conjecture.

The Siricoidea of the Chalastogastra, on account of their legless, eruciform, lignivorous larvæ and reduced wing venation appear to have been derived from some sort of ancestor with a caterpillar-like larva having the more complex wing-venation seen in the saw-flies or Tenthredinoidea. So far as is known, no member of either group is parasitic. Until recently the family Oryssidæ has been regarded as a degenerate group quite closely allied to the Siricidæ. Rohwer has, however, shown that they are really very different and finally (Rohwer, '17) regarded them as a distinct suborder of Hymenoptera. It seems reasonable to suppose that they have Siricid-like ancestors, and as they are now known definitely to be parasitic on the larvæ of wood-boring Coleoptera, it ap-

[2] This matter, as well as several others discussed in the present paper, have been recently dealt with by Picard in a publication (La faune entomologique du figuier, '19) which I unfortunately did not see until too late to refer to it in the text of this article. Picard's "Considérations sur les parasites," pp. 166–172, are of extreme interest.

pears that we have in them the most primitive parasites in the order Hymenoptera. The hosts of the Oryssidæ consist partly, although probably not entirely, of Buprestidæ, which paleontology shows to be an ancient family. Handlirsch ('08) has even gone so far as to suggest that the parasitic Hymenoptera may have been derived from the Jurassic Pseudosiricidæ which no longer laid their eggs in wood, but in the eggs of beetles occurring in the wood. This is entirely speculative and so I think must be at the present time any suggestions as to how the Oryssidæ, or the Ichneumonidæ, which Handlirsch had in mind, became parasitic. That their larvæ first found and fed upon their hosts after hatching seems much more probable. It must be said, however, that predatory or carnivorous Chalastogastra are not known among living forms, except certain adult sawflies which fed in this way (cf. Mrázek, '09). From this point onward we have little trouble in tracing the probable origin and relationships of the Ichneumonoid families as I have attempted to do in a previous paper (Brues, '10). Thus the Stephanidæ are structurally primitive and strikingly like the Oryssidæ in the peculiarly horned head which had been remarked on before the habits of the Oryssids were known. On account of the presence of a costal cell in the wing, the polymorphic family Evaniidæ is necessarily also more primitive than the Ichneumonidæ or Braconidæ, and, through one subfamily, the Fœninæ, resemble the Stephanidæ as has been already noted by Bradley ('08). Some Braconidæ, the Stephaniscinæ, Spathiinæ and Hormiinæ are much like Stephanids, so much so that it is difficult to believe that they are not directly derived from them. One other family, the Capitoniidæ, recently segregated from the Braconidæ, appears to be very definitely related to the more generalized Evaniidæ (Aulacinæ). Omitting in this brief consideration several less pertinent families, and ignoring other recently segregated ones, we have left only the Ichneumonidæ, related possibly through the Alysiidæ to

the Braconidæ. Structurally this relation seems plausible, but as the Alysiids attack almost exclusively the highly specialized Diptera it is very difficult to regard them as closely related to the ancestors of the Ichneumonidæ, so highly diversified in habits and structure. The latter then are not so easily derived and may go back to Evaniid-like forms.

One extremely interesting fact in connection with the primitive families of parasitic Hymenoptera is their association with wood-boring insects. Thus the Oryssidæ, the most generalized group of Evaniidæ, the more primitive Braconidæ, many of the structurally primitive Ichneumonidæ, and the Capitoniidæ are restricted to hosts having such habits. This shows undoubtedly that such habits have not easily been changed and that similarity of host-habits is an important factor in determining what insects may be attacked. This supports strongly our thesis of the interrelation of taxonomy and habits.

In connection with the parasitism of certain chalcisflies, the French entomologist Marchal ('98) discovered, some years ago, a most anomalous method of precocious multiplication which he designated as polyembryony or germinogony. In species exhibiting this phenomenon, the embryo becomes dissociated into a large number of parts, and from the numerous germs thus produced there is formed a veritable swarm of minute parasites, the extent of which is limited only by the available food supply in the host. Marchal's first observations have been much extended since by himself and numerous other workers, and the same condition has been found to exist in many other Chalcidoidea and also in the Serphoidea (Marchal, '03). It has recently been recognized in another widely different family, the Dryinidæ by Kornhauser ('19) and probably occurs sporadically in several other parasitic families, although I believe no other cases have been absolutely substantiated. From the regular association of numerous individuals in single hosts in the case of *Microgaster*, allied genera of the Braconidæ,

and in *Sphæropyx* (Cushman, '13), in a few Ichneumonidæ (*e.g.*, species of *Cryptus*) and in a few Bethylidæ, it would seem likely that they also are polyembryonic.

The widespread occurrence of germinogony and its apparently erratic distribution show that it can be of no general taxonomic interest at least as an aid to classification. It is indeed quite the opposite, for the development of the egg in the process of fragmentation is so similar in the Chalcidoid and Serphoid that we might be led to believe it of common origin. As their ancestors were undoubtedly not polyembryonic, such can not be the case and the process must have originated independently, just as it has in several totally unrelated animals like certain annelids (*e.g.*, *Helodrilus*) which exhibit it in an imperfect condition (Weber, '17) and in the armadillo (Newman and Patterson, '10) among mammals where it has become completely established. A quite similar modification of development is seen in the formation of the rediæ in the sporocysts of Distomes. Still similar, but delayed until the larval stage, is the process of pædogenetic multiplication in the Cecidomiid fly Miastor (*cf.* Felt, '11), well known to all entomologists.

It appears from any general survey of the habits of the parasitic Hymenoptera that we find certain taxonomic groups of host commonly attacked by discrete groups of closely related parasites. It is natural that such combinations should impel our attention, as they may be fitted with the least effort into a classified scheme, and furthermore their mere recurrence is sufficient to indicate that they are not due merely to chance.

The following list includes a few striking instances of this sort drawn at random from widely separate sections of the order:

Parasites	Hosts to Which They are Restricted
Families	
Alysiidæ	Dipterous larvæ.
Trigonalidæ	Vespidæ.

Subfamilies	
Evaniinæ	Cockroaches and their oöthecæ.
Ichneutinæ	Saw-fly larvæ.
Genera	
Polygnotus	Cecidomiid larvæ.
Coccophagus	Soft scales.

If we should reverse the order of the above list and attempt to tabulate groups of related hosts that are affected only by certain groups of parasites we should have great difficulty in finding examples. This, of course, is to be expected on account of the passive condition of the host and the active rôle of the parasite, whereby it first came to infest some certain kind of host. Inheritance of such specific instincts over long periods of time, during which groups were becoming differentiated, will lead naturally to the evolution of groups of parasites attached to groups of hosts which have meanwhile been developed. Such reasoning appears to be sound and may explain some of the conditions tabulated above.

I think, however, that there is a deeper basis than this, and that we can not fully understand such combinations without inquiring into the actual physiological relations between host and parasite.

It has been customary among entomologists to place great emphasis upon the maternal instinct of invariable selection as determining and restricting the range of hosts affected by specific parasites. Among zoologists who deal with other parasites, particularly Protozoa and lower invertebrates, no such idea has ever been entertained, as the parasite plays a passive rôle in attaining its host. The malarial parasite is ingested by all insects that suck human blood, but is able to continue its parasitic life only in certain particular mosquitoes. Similarly, a certain Cestode worm parasitic in birds has as intermediate host, the garden slug, from which the definitive host obtains it by eating the slug. That this Cestode does not occur in other hosts that may eat infected slugs is a phys-

iological matter and is always regarded as such by helminthologists who encounter many instances of this kind. On account of the definite requirements of such parasites, Cobb ('04) suggested some years ago that they might give valuable clues to the taxonomic affinities and physiological peculiarities of their hosts, the latter particularly in cases where there is a wide range of hosts.

In insects, and, quite fortunately for the present discussion, in the parasitic Hymenoptera, there are available some extremely pertinent observations made by Timberlake ('12) relating to the fate of eggs in the bodies of host insects in which they do not normally develop. His experiments were made with an Ichneumonid, *Linnerium validum,* commonly parasitic in caterpillars of the fall web-worm. This parasite will also oviposit in larvæ of various other moths, when persuaded to do so in captivity, by depriving it of its normal host; but it can not complete its development in the experimental hosts. This is due to the death of the young larvæ, which succumb to the reactions of the host soon after hatching, or possibly in some cases even before hatching. The antagonistic action of the tissues of the host is manifest by the accumulation of amœbocytes about the unwelcome objects. In one other abnormal host, the tent-caterpillar, this *Linnerium* may survive and complete its transformations, but there is a high mortality among the parasites, for many are destroyed by the host.

These experiments show very clearly why this parasite is restricted to certain hosts and, from the nature of the reaction, which is so similar to that exhibited by animals in general toward microorganisms and other foreign materials, there is little reason to doubt that insects usually react in this fashion. This also furnishes an explanation for the continued restriction of parasites to specific hosts, based upon natural selection, since individuals choosing unsuitable hosts will suffer a very material reduction in the number of their immediate progeny. This is, I think, especially important, as it takes much of the burden from

the already greatly strained principle of the fixity of instinct in the imaginal insect.

It also aids greatly in understanding the relation previously referred to, where extensive groups of parasites attack discrete groups of host. Adaptation to one host means ordinarily greater physiological suitability for another closely related host than for a widely different one. This, no doubt, applies to cases like the Alysiid parasites, for here the series of hosts, while quite uniform, is so extensive that it can not be explained on the slowly acting basis of concomitant differentiation of the hosts and parasites.

Instances, like one cited by Pierce ('08) where several species of parasites suddenly became abundant enemies of the boll-weevil due to the scarcity of their more favored hosts, must depend upon selection, as suggested above, leading to the rapid improvement of partial adaptations.

We have already referred to the fact that the parasitic Hymenoptera, and quite generally also most parasitic insects, attack other insects, and pointed to this as a characteristic more or less peculiar to insect parasitism or at least to its most prevalent types.

The attachment to closely related animals as hosts is shown still more clearly in Hymenoptera that are secondary parasites on parasitic species of the same order, of the same family, or even of related genera. This phenomenon is not restricted to Hymenoptera, but is most extensively exhibited by them. Thus certain genera of Ichneumonidæ, Braconidæ, and Chalcidoidea develop regularly in the larvæ of primary parasites which become established in a free living host.

Secondary parasites are not absolutely distinct from primary ones in some individual cases, for this relation is known to be facultative in a few species of Hymenoptera which develop in either way. In 1903, Fiske ('03) showed from careful breeding experiments that certain Ichneumonidæ of the genus Itoplectis may be either pri-

mary or secondary parasites of the tent-caterpillar, attacking a member of their own family in the latter case. Since then other examples have come to light, but they are by no means common. Another fact which is significant in connection with secondary parasites is that they are very generally much less particular than primary ones in restricting themselves to a small series of hosts.

In searching for the origin of secondary parasitism, it is certain that it must be derived from the primary form, since it is naturally dependent upon the latter for its mere existence. The only other possibility appears to be the assumption that the primary parasites were free-living forms when first parasitized, and that they have since developed parasitic habits of their own. As the secondaries are frequently structurally reduced such a supposition appears still more improbable.

If, then, secondary parasites are derived from primary ones, what can have caused them to desert their free-living hosts? We have already seen how the restriction of hosts among primary parasites seems to have a physiological basis, in that the reaction of the tissues of the host has been shown (Timberlake, '12) to eliminate parasites not adapted to it. In attacking insects very closely related to themselves parasites should stand a much better chance for successful growth, as the physiological antagonism of all animals toward closely related forms is much less than that toward very different ones. Young larvæ of parasitic species should therefore meet with less difficulty in developing in the bodies of related forms, and secondary parasitism might arise with little difficulty when eggs were placed in another parasite rather than in the body cavity of the free-living host. This explanation may account for the prevalent type of hyperparasitism, but not for cases like that of the Chalcidid *Dibrachys* which attacks Hymenoptera and Diptera alike. This may simply be a case of great adaptability in certain species like some mentioned in connection with pri-

mary parasitism, although it may depend upon a general similarity in the tissues of all entomophagous parasites, or a less aggressive condition of the tissue in parasites due to their generally secluded and protected environment. As the latter condition seems not unlikely, it probably acts regularly to make hyperparasitism an easily acquired characteristic.

Striking divergencies, like the following, noted by Swezey ('08), are of interest in this connection. In his studies of Dryinid leaf-hopper parasites, he found a Ceraphronid parasitic on a species of the related Dryinidæ, although the group normally and abundantly parasitizes entirely different types of insects.

The adaptation of animals and plants in conformity with the demands of diverse environmental conditions is now an axiom among biologists. From its manifestations it is evidently a physiological adjustment which leads secondarily to structural changes, and many convergences in form and function are traceable to it. On account of the close interdependence of plants and insects it appears, in some instances at least, to exert an indirect influence upon phytophagous insects (Brues, '20), whereby a species may feed rather indiscriminately on herbs, and another on woody plants, but not upon the two in combination.

In the case of parasitic Hymenoptera there are many instances which might be cited where environment appears to have exerted a direct influence upon the acquisition of host relations and others where we must, I think, believe the influence to be indirectly related to the environment through a second insect, the host. This rather obscure statement may be clarified by a few examples. From what we have said in connection with hyperparasitism, it seems quite clear that a species which may assume the rôle of either a primary or secondary parasite, responds quite directly to the environment, in this case the primary host, which may be either sound or already infested by a parasite which is in turn at-

tacked. This influence seems to be a rather direct one. On the other hand, I may quote from a previous paper (Brues, '08) the following: "The European Chalcid-fly, *Ormyrus tubulosus*, has been minutely studied by Mayr, who has bred it from no less than 27 species of Cynipid galls, and I have from Massachusetts what is apparently the same species, bred from about half as many North American species by the late Dr. M. T. Thompson. The galls formed by the various hosts of this species are many of them entirely dissimilar in form, the only resemblance between them, aside from their gross gall-like form, being their more or less uniform habitat attached to twigs and leaves." Howard ('91) mentions *Enrytoma rosæ* as having over 50 cynipid hosts. A range of hosts of this sort appears to be due not directly to the environment of the host, but to the similar physiological condition of the various Cynpids themselves, which, as we have already said, are closely confined to a very narrow range of food-plants.

The great difficulties occasionally imposed upon parasites in attaining their hosts may be purely a matter of environment, as illustrated by the following considerations.

An interesting series of parasitic Hymenoptera are those which prey upon aquatic insects. In several well-known cases, the behavior of the adult parasites has become so profoundly modified that the females not only enter the water in search of their hosts, but they may be, occasionally at least, accompanied by the males. The first observation of this sort was made nearly a century ago by Francis Walker ('36) on *Agriotypus*, and the well-known observer Sir John Lubbock ('63) later gave an account of the habits of two aquatic Chalcis-flies in which he describes the actual process of swimming. One species, the Mymarid (*Cataphractus cinctus*) makes use of its ciliated, paddle-shaped wings for this purpose, while the other, a Trichogrammid (*Prestwichia aquatica*) propels itself by means of the legs. Numerous other contribu-

tions, notably those of Von Siebold ('58), W. Müller ('89), Marchal ('00), Rousseau ('08), Heymons ('08), Schulz ('07, '10[a], '10[b]), and Matheson and Crosby ('12), have added much of interest, not only in bringing to light aquatic members of several families, but in determining some of the host species upon which they prey. In many cases the adaptation to aquatic life is not so perfect as the cases just mentioned, although several other species are known to swim readily, using either the legs or wings, which usually show modifications adapted to such behavior.

In view of the frequent occurrence of aquatic imaginal forms in other orders of insects such as the Coleoptera and Hemiptera, it is perhaps not surprising to find certain parasitic Hymenoptera adopting this habitat. Viewed more in detail, however, the matter is quite a different phenomenon. Such Coleoptera as Gyrinidæ, Hydrophilidæ, Dytiscidæ, etc., are uniformly aquatic in both preparatory and imaginal stages, and such is also true of the brachycerous Hemiptera. All of these insects are highly modified to conform with their aquatic environment, particularly in reference to the functions of locomotion and respiration.

In the aquatic Hymenoptera, a series of families is represented and only a comparatively small number of genera are included. The structural modifications are far less profound, indeed they frequently represent very slight changes. They are more closely parallel to the natatorial habit shown in isolated genera such as the rice water-weevil, *Lissorhoptus simplex*, a beetle that has become aquatic and oviposits in the roots of the rice plant (Tucker, '12). It has been shown experimentally by Szymanski ('18) that many terrestrial insects may be induced to swim if submerged and we may easily suppose that the truly aquatic habit of the parasitic Hymenoptera just mentioned may have arisen through the seeking of their hosts in aquatic plants, first at or above the surface of the water, and later through a search for further indi-

viduals below the surface. Even memory could easily play a part here, if the host were submerged during the development of the parasite, and the latter emerged as an adult below the water, from which it must escape by locomotion through the water.

In the case of aquatic Hymenoptera, it is readily seen that we can not correlate taxonomy with habits according to any generalized scheme, although the several genera show structural characters associated with their unusual habits. Most striking is the number of Mymaridæ and Trichogrammatidæ included, minute insects whose wings are naturally well suited for swimming.

Frequently a secluded habitat acts as a powerful factor in restricting the kinds of parasites that can attack certain types of hosts. Thus, wood-boring insects can be reached only by species provided with long ovipositors. Such restrictions are clearly defined and many other examples might be cited. Partial inaccessibility of the host may even occur in the case of parasites otherwise well suited to their host, as for example in the case of a common parasite of the eggs of the gipsy moth, which is able to oviposit only in the eggs occupying a superficial position in the egg-mass of the host. Sometimes difficulties may be overcome by the presence of an active first-stage larva. This may exhibit most extraordinary behavior as has been described by Smith ('17) in the Chalsis-fly, *Perilampus*. Here the *Perilampus* egg is deposited and hatches away from the body of the host as a planidium which later attaches itself to the host and remains there till the host completes its growth, after which the planidium begins its parasitic life. A second species that is a hyperparasite seeks out the primary parasite in the caterpillar host through which it bores its way and there awaits the exit of the primary parasite before proceeding with its development.

Again, the female of some egg-parasites attach themselves to individuals of the host species and are thus carried to the place where the eggs within which they will

develop are to be deposited. Certain Chalsis-flies and Serphoids have adopted this curious method of transportation (Brues, '17) which occurs sporadically in diverse insects (Banks, '11). The way in which many modifications of this kind appear in similar form makes it impossible to consider them as guides to taxonomic affinity. The elongated ovipositor, the active first-stage larva, and many other adaptations for attaining the host are of course good taxonomic characters, but they reappear independently in more than one group, and can be used only in combination with characters of less vital importance to the animal, to characterize completely any extensive groups. Nevertheless, the lengthened ovipositor can be used to separate numerous families and smaller groups in the parasitic Hymenoptera and as it bears a certain relation to habits, the latter are thus reflected in taxonomy on a structural basis. However, the habits of many such insects do not seem to require such a long ovipositor and represent not the primitive habit for the group, but recent modifications which break down the homogeneous correlation of structure and habits.

Closely connected with the specific association of natural groups of hosts and parasites is the great variation shown by different parasites in the number and diversity of the species that serve as their hosts. Just as we can find among phytophagous insects, omnivorous forms, strictly monophagous ones, and all intergrades between the two, so there exists among parasites an almost equally varied series of associations with one, several or many hosts.

Although parasitic Hymenoptera are so abundant, both in species and individuals, their food habits are not so easily observed as those of plant-eating insects and our knowledge concerning them is far less complete. The large number of secondary parasites also lead to confusion, as these may not always be distinguished on a structural basis.

If parasitism demands a nice physiological adjust-

ment, we might expect to find that egg-parasites affecting the organism at an earlier and less highly differentiated stage of ontogeny, are more catholic in their tastes. This is, however, not borne out by observation to any extent, and egg parasites are usually as closely restricted to particular hosts as their relatives who confine their attention to larval insects.

Small size is a prerequisite of all true, internal egg-parasites except a few that occur in the oothecæ of cockroaches, where the comparatively large species of *Evania* undergo their development. Some parasites oviposit in the host-egg, but live at the expense of the larva; they are, except in polyembryonic forms, larger, and not classed as egg-parasites.

On the basis of size, then, practically all egg parasites are either Chalcidoidea or Serphoidea and this habit characterizes a number of families, and smaller taxonomic groups (*cf.* Girault, '07, '11). Among them the strange, tropicopolitan genus *Podagrion* attacks only the eggs of Mantidæ. The large cosmopolitan genus *Telenomus* occurs in the eggs of various insects, mainly Lepidoptera while the very similar genera *Phanurus* and *Trissolcus* are restricted to eggs of Tabanidæ and Pentatomidæ. Again, *Scelio* and several related genera attack only the eggs of the Orthoptera Saltatoria. Thus, if used with due caution, egg-parasites are in the main illustrative of close correlation between the taxonomy of host and parasite in spite of the fact that we may naturally regard insect eggs as more similar *inter se,* than insect larvæ.

It is true that the ubiquitous little *Trichogramma* affects eggs of several orders and many families of insects, but like other less conspicuous examples, it stands quite apart from its commonplace associates.

With their larger and more variable size, and great diversity in habits and structure, larval insects present a correspondingly varied series of opportunities for parasites. We find also that practically no genera are known

to parasitize both eggs and larvæ, although the polymorphic and widespread *Eupelmus* among the Chalcisflies appears to be an exception. As the eggs and larvæ of many insects frequently occur together at the same time, this fact is rather surprising and shows that the parasitic association must depend greatly upon gross form, as well as upon the factors of environment and specific physiological reactions, which we have already mentioned. One case which comes to my mind in this connection is quite instructive and there are no doubt others of a similar nature. All the several genera included in the Evaniinæ are, as previously mentioned, parasites in the egg-cases of cockroaches, with the exception of a single reliable record (Picard, '13) of the rearing of *Zeuxevania* from the body of the blattid itself. Quite likely the future may bring forth other similar observations on Evaniines, but this one shows that parasitism has been transferred to the cockroach from the oötheca, which is of course carried about by the female for some time before deposition.

Larval parasites have been more extensively reared than those living in eggs and their habits are consequently better known. Many observations upon individual species of hosts show that the larval stages harbor a far more extensive series of parasites (*e.g.*, Howard and Fiske, '12) than the eggs or pupæ, while hymenopterous parasites of the adult are almost unknown. Among larval parasites it is easy to recognize two general series, so far as the number of hosts utilized. Some species are very conservative in this respect and others extremely versatile. These two terms are equally suitable for genera and larger groups, and the difference is more important when it involves all or most of the species of quite extensive groups. Thus the highly modified members of the family Dryinidæ (Perkins, '05) are restricted to several families of Homoptera. A few which parasitize Membracids are insects of quite ordinary appearance, but the remainder affecting Tettigoniellids and

Fulgorids have the fore tarsi of the females misshapen to form chelæ or pincers, by means of which they cling to their host. Such structures are elsewhere unknown among insects. The group has become highly specialized, apterous in several genera, and has probably reached the end-stage in its evolution. Like all creatures which have attained this condition, it shows no further adaptiveness in habits. This is a clear-cut case of correlation between habits and taxonomic affinities.

Versatile groups naturally include large numbers of genera and species with varied habits which enable them to grasp every opportunity to earn (or, in the case of parasites, to steal) a livelihood. Numerous species and varied habits, are as inseparable as form and function. The former binary involves an added series of factors, since any group of insect parasites comes into keen competition with the members of other groups as it reaches out for new hosts. Some have spread widely among hosts of very similar types, restrained by some insuperable obstacle, probably physiological in nature, from attaching themselves to strange insects. They show a correlation between habits and structure. Others have broken their fetters more quickly and completely, and adaptations in habit have far outstripped structural modifications, resulting in natural taxonomic groups which show only imperfectly such correlation.

The climax in this direction is reached by certain groups which have cast aside parasitism entirely and become phytophagous. This has occurred independently in several families of Chalcis-flies, a group in which the struggle for existence must be very severe. One of these aberrant series, *Megastigmus* and its allies (Crosby, '13) feed within the seeds of plants, mainly those of various trees, upon a rich protein diet, probably similar to that of their entomophagous forebears. Another, *Isosoma* and its allies (Howard, '91 and '96; Phillips and Emery, '19) occur in far less delectable vegetable tissue, such as the culms of grasses in which they sometimes cause

galls. A third (Mayr,' '05), including some genera related to the remarkable parasitic *Perilampus,* which we mentioned a few moments ago, produce conspicuous galls on certain plants.

The production of galls by the phytophagous Chalcids is quite suggestive, since many forms related to Isosoma (*Harmolita*) are parasites of gall-making Cynipids. *Megastigmus* also belongs to a group including many parasites of Cynipids. Since we do not know exactly how galls are formed, however, the matter can not be profitably discussed at the present time.

Although they may not aid us greatly in formulating any general causes leading to divergence in habits among related forms, I should like to append a few observations made by various entomologists which suggest a variety of factors.

The effect upon the parasite of almost complete elimination of a host through excessive parasitism has often been commented upon by entomologists. An especially clear case has been described by Aldrich ('12) where an invasion of the western pine-butterfly was suddenly checked by *Theronia fulvescens.* The parasite then found it necessary to eke out an existence from scattering and less suitable forest insects and under such stress, selection must be very keen (cf. p. 147). Complete parasitism of 100 per cent. of the related cabbage butterfly by *Apanteles glomeratus* has also been reported by Chittenden ('05).

Errors or aberrations of instinct have also been occasionally observed. Thus Marchal ('07) saw a Chalcidid parasite of coccinellids (*Lygellus*) repeatedly oviposit in the pupal exuvia when living material was not available. Still more incongruous is the behavior of *Trichogramma* observed by Holloway ('12) who found this insect actually ovipositing in small globules of partly solidified plant juice on the foliage of okra plants. One of the common hosts of this egg-parasite, the cotton boll-worm, frequently oviposits on the leaves of okra and the globules

were evidently mistaken for moth-eggs. Premature oviposition is generally attributed to physical necessity in relieving the pressure in the body, but here at least it is accompanied by the outward appearance of instinct. Whether this Chalcid tasted the strange new host is not stated, but it is a common procedure among Chalcids (Howard, '10) to tap the host with the ovipositor, and to lap up the exuding body-juices quite independently of egg-deposition. What her reasons for this may be are obscure; possibly it is to test the suitability of the host; perhaps to secure food, or she may even retain a specific appetite for the kind of food consumed in her earlier days.

Marchal, Vayssière ('07) and Loiselle ('08) have commented upon the retarded development observed in certain Ichneumon-flies whereby emergence of some individuals was delayed a year. Such occurrences might serve to bridge over the gap of a season when host insects were scarce; on the other hand, if the time were not exactly twelve months it easily might lead to a new "trial" association in the absence of the proper host at that season.

That these factors might lead to divergence in habits, I can not doubt, but hesitate to apply them to any concrete cases of aberrant habits.

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ONCE MORE THE SUCKING-FISH

PROFESSOR LEO WIENER

HARVARD UNIVERSITY

IN 1919, while Professor E. W. Gudger's excellent series of articles "On the Use of the Sucking-fish for Catching Fish and Turtles" appeared in THE AMERICAN NATURALIST, I was at work on my first volume of "Africa and the Discovery of America," where I had to touch on the remora story in the early voyages to America, in order to show that they were all a myth, based on the literary influence of Odoric of Pordenone on Columbus. As my sources were naturally of a different character from those of Professor Gudger, who was chiefly interested in the zoological side of the question, I was able to supplement his thorough discussion with a number of new data, which the zoologist will not consider to be amiss.

The remora was dimly known to all the Arabic voyagers. We meet with it in the middle of the ninth century, in the very beginning of the "Chain of Chronicles."[1]

> In the Indian Ocean there is a fish, twenty cubits in length, in whose belly there is a fish of the same kind, in whose belly there is similarly a third fish. All these fishes are alive and moving. This large fish is called *al-wāl*. In spite of its size it has for its enemy a fish only a cubit in length, called *el-leshek*. When the large fish becomes angry and attacks the other fishes in the sea and maltreats them, the little fish takes charge of him: it attaches itself to the root of his ear and does not let go of him until he is dead. The little fish also attaches itself to boats, and the large fish dares not approach it, because of the fear with which it inspires him.

[1] M. Reinaud, "Relation des Voyages faits par les Arabes et les Persans dans l'Inde et à la Chine dans le IX^e^ siècle de l'ère chrétienne," Paris, 1845, Vol. I, p. 2 f.

This account is obviously an exaggeration of some story about the shark, but *wāl* was soon identified with the whale, as appears from the later Arabic sources. A century later Mas'ūdī wrote:

> There is a fish in this sea called el-Owāl, which is from four to five hundred 'Omari cubits long; these are the cubits in use in this sea. The usual length of this fish is one hundred perches. Generally the head of the whale is out of the water; and when it powerfully ejects water, it gushes into the air more than one bowshot high. The vessels are afraid of it by day and night, and they beat drums and wooden poles to drive it away. This fish drives with its tail and fins other fish into its open mouth, and they pass down its throat with the stream of water. When the whale sins God sends a fish about one cubit long called *esh-shak* (*al-leshck, as-sal*), it adheres to the root of its tail and the whale has no means to make itself free from it. It goes therefore to the bottom of the sea and beats itself to death; its dead body floats on the water and looks like a great mountain. The fish called *esh-shak,* adheres frequently to the whale. The whales notwithstanding their size, do not approach vessels; and they take flight when they see this little fish, for it is their destruction.[2]

Idrīsī merely said that in the Sea of Oman the *wālī,* which is of white color and one hundred cubits in length, is usually accompanied by the small *leshek,* which kills it.[3] Ad-Damīrī definitely identifies the large fish with the *bāl,* the whale.

> When it begins to tyrannize the other animals of the sea, God sends a fish about a cubit in length, which attaches itself to its ear, and the *bāl* seeing no means of freeing itself from it, goes down to the bottom of the sea and strikes its head on the ground until it dies, after which it floats on the top of the water like a big mountain, and the men on the East Coast of Africa are generally on the look-out for it. When they find it, they plunge harpoons on it and drag it to the shore where they cut open its belly and take out of it ambergris.[4]

The important point in all these stories, which obviously emanate from the same original account, is that

[2] A. Sprenger, "El Masʿūdī's Historical Encyclopedia, entitled 'Meadows of Gold and Mines of Gems,'" London, 1841, Vol. I, p. 263 f.

[3] P. A. Jaubert, "Géographie d'Edrisi," Paris, 1836, Vol. I, p. 63.

[4] Ad-Damīrī's "Hayāt al-Hayawān" (a zoological lexicon), translated by A. S. G. Jayakar, London, 1906, Vol. I, p. 237.

the remora is found off the coast of Zanzibar, where it is in the same way connected with the catching of large fish. But we have a circumstantial report of the employment of the sucking-fish in the catching of sea turtles in João dos Santos' "Ethiopia oriental," which was published in 1609:

> The fishermen kill turtles at sea [along the coast of Mozambique] in a strange manner. First they catch in certain parts of the sea among the rocks near the coast a kind of fish two spans in length, called by the Moors *sapi*, which is as much the enemy of the turtle as the ferret is of rabbits. The *sapi* has a very dark grey skin inclining to black, and a long thin head ending in a snout similar to that of a pig. Its neck is about half a span long, on the back of which is a shell of the same length and three fingers wide, which is formed of hard and porous furrowed skin with which it clings to the stones as leeches do, and it has the same faculty of sucking blood. For this reason when it meets a turtle it attacks it and wounds it in the neck or legs with this shell, and sucks its blood until it is satiated, leaving the turtle nearly dead, it being unable to resist or get away, as it is large and unwieldy and the *sapi* very nimble.
>
> When the fishermen have caught some of these *sapis* they put them in a basin of salt water and take them in the boat with them. They tie a long line to their tails and then put out to sea in search of turtles, which usually swim on the surface of the water. When they catch sight of a turtle they throw out the fish fastened by the tail, as one lets loose a ferret in a leash after a rabbit, and the fish immediately attacks the turtle with as great force as if it was free and had received no harm from the hook with which it was caught, or as if it was not itself a prisoner. When it reaches the turtle it fastens on it so tightly that it never looses its hold, and as soon as the fishermen feel that it had done so they pull in the line and draw it over the water without its loosening its hold, and the turtle, although very big and heavy, is so dominated and tormented by the fish that it does not fight with it, but lets itself be carried off easily because of the pain it suffers while they are pulling it in, as at that time the fish grips it much tighter. Thus the turtle is brought to the side of the boat, when the fishermen quickly seize it in their hands and lift it in, and the fish they put back into its basin. In this manner they catch a number of turtles.[5]

In the Bantu language of Zanzibar we have "*kassa*, turtle; the *kassa* is caught by means of the *taza* fish,

[5] George McCall Theal, "Records of Southeastern Africa" (London), 1901, Vol. VII, p. 325 ff.

which the fishermen carry alive with them; when they see a *kassa*, they let the *taza* go after it to stick fast to the *kassa*; when the *taza* has seized it, the fisherman throws a harpoon and takes the *kassa* out of the sea, the *taza* letting go instantly when exposed to the air.''[6] The same dictionary gives *tasa* "a kind of fish which serves as a bait for turtles,''[7] but the other dictionaries give for it *chazo*, which name is also recorded by Professor Gudger. *Kassa* for "turtle" is of extremely wide distribution and is not primarily a Bantu word, although it is also found as *kasi* in Tete, that is in the region to which dos Santos refers.

The oldest forms on record for this word are Sanskrit *kacchapas*, *kaçyapas*, Avestan *kasyapa*, hence Persian *keshef*, Afganistan *kasph*, Singhalese *keshew*, Hindustani *kacchua*, *kaccha*. It is therefore certain that the turtle fishing was brought to the shores of Zanzibar from somewhere in the Indian Ocean. This is in keeping with the frequently recorded tortoise-shell trade in the Indian Ocean, but "opposite the Ganges there is an island in the ocean, the last part of the inhabited world toward the east, under the rising sun itself; it is called Chryse, and it has the best tortoise shell of all the places on the Erythraean Sea.''[8] The Chryse Island has been identified with the Malacca peninsula,[9] hence the origin of the practise of catching turtles with the remora is most likely to be referred to the East Indies, whence it traveled eastward, to the Torres Strait and Melanesia, and westward to the eastern shores of Africa.

The earliest definite reference to the remora fishing is contained in a version of the cormorant fishing, as told by Odoric of Pordenone and for the first time printed in Ramusio in 1574, although it can be shown that it was

[6] L. Krapf, "A Dictionary of the Suahili Language,'' London, 1882, pp. 130 f.

[7] *Ibid.*, p. 362.

[8] W. H. Schoff, "The Periplus of the Erythraean Sea,'' London, 1912, p. 48.

[9] *Ibid.*, p. 259.

already in existence in the fourteenth century. Odoric of Pordenone told how he came

to a certain great river, and I tarried at a certain city (called Belsa) which hath a bridge across that river. And at the head of the bridge was a hostel in which I was entertained. And mine host, wishing to gratify me, said: "If thou wouldst like to see good fishing, come with me." And so he led me upon the bridge, and I looked and saw in some boats of his that there were certain water-fowl tied upon perches. And these he now tied with a cord round the throat that they might not be able to swallow the fish which they caught. Next he proceeded to put three great baskets into a boat, one at each end and the third in the middle, and then he let the water-fowl loose. Straightway they began to dive into the water, catching great numbers of fish, and ever as they caught them putting them of their own accord into the baskets, so that before long all the three baskets were full. And mine host then took the cord off their necks and let them dive again to catch fish for their own food. And when they had thus fed they returned to their perches and were tied up as before. And some of those fish I had for my dinner.[10]

This is followed by another kind of fishing:

The men this time were in a boat, wherein they had a tub of hot water; and they were naked, and had each of them a bag slung over his shoulder. Now they dived under water (for half a quarter of an hour or so) and caught the fish with their hands, stowing them in those bags that they had. And when they came up again they emptied the bags into the boat, whilst they themselves got into the tub of hot water, and others went in their turn and did as the first; and so great numbers of fish were taken.[11]

The second kind of fishing is interesting from the fact that it was much earlier told by Idrīsī as in use at Zanzibar.

These people (at Meduna) fish in the sea without boats. They fish by swimming, with small nets spun from grass and manufactured by them. They tie these strings to their feet by means of knots which they hold in their hands, they draw the strings of the net together the moment they feel that the fish have entered, and this they do with an art in which they excel, and with rules in which they have long experience.

[10] Sir Henry Yule, "Cathay and the Way thither," London, 1913, Vol. II, pp. 188 ff.

[11] *Ibid.*, pp. 190 f.

To attract the fish they use land reptiles. Although they live in a state of great distress and misery, these people (God loves those who reside at their family hearths) are satisfied with their lots and with what they have. They are under the government of Zanzibar.[12]

Yule[13] cites Fortune and Dabry for the same custom in China, which once more shows the wide distribution of identical maritime customs from Zanzibar to China.

The first kind of fishing has undergone all kinds of changes in the very earliest Odoric manuscripts. Sir John Mandeville, who cribbed so much out of Odoric, tells of a fish-otter, instead of a cormorant, as the animal with which fish are caught.

> In that country there be beasts taught of men to go into waters, into rivers and into deep tanks for to take fish; the which beast is but little, and men clepe them *loirs*. And when men cast them into the water, anon they bring up great fishes, as many as men will. And if men will have more, they cast them in again, and they bring up as many as men list to have.[14]

It is interesting and important to observe that the Italian version of Sir John Mandeville which came out in 1491,[15] that is, one year before the discovery of America, has the same story, the French term *loir* for "otter" being here rendered by *udria*. The Latin version, of about 1500,[16] simply says:

> Tamed water dogs whom we call *luteres*, are here aplenty; every time they are sent into the river, they bring out fish.

The substitution is everywhere from Vincent of Beauvais, who in his "Speculum naturale," XX, 89, tells of the same fish-otters with which fish are caught, but the substitution is unquestionably older than Sir John Mandeville's, who would not have omitted the strange story of the cormorant if he had found it in his copy of Odoric.

[12] *Op. cit.*, pp. 55 f.

[13] *Op. cit.*, p. 191.

[14] "The Travels of Sir John Mandeville," London, 1900, p. 136.

[15] "Tractato delle piu maravigliose cose e piu notabili," Venice, Nicolaus de Ferrariis, 17 Nov., 1491, cap. CXLVII.

[16] "Johannis de Montevilla Itinerarius in partes Iherosolimitanas," cap. XXXI.

The Latin version of Odoric has the old cormorant story[17] where the bird is called *mergus*, while in the Italian version it is called *smergo*.[18] The usual Italian names for the cormorant which Odoric must have known, are also *mergo, maragone*,[19] so that the Latin *mergus* is formed from the Italian *mergo*. Curiously, there are two versions of Odoric in Ramusio. In the first the whole cormorant fishing episode is omitted, while the second has a totally different account. Here we read:

> Mine host . . took us to one side of the bridge where the river was wider, and there we found many boats, and there was one of them employed in fishing by aid of a certain fish called *marigione*. The host had another such, and this he took and kept it by a cord attached to a fine collar. And this indeed is a creature that we have seen in our own seas, where many call it the *sea-calf*. It had the muzzle and the neck like a fox's, and the fore paws like a dog's, but the toes longer, and the hind feet like a duck's, and the tail with the rest of the body like a fish's. Mine host made him go in the water, and he began to catch quantities of fish with his mouth, always depositing them in the boat. And I swear that in less than two hours he had filled more than two big baskets.[20]

It is clear that the description of the *sea-calf* is an exaggeration of that of the fish-otter, which is in Arabic called "fox of the water" or "dog of the water." Hence there is most likely here an Arabic influence which caused the substitution. And the reference to a fish *marigione*, which was kept by a cord attached to a fine collar, is similarly an attempt to bring the cormorant story in keeping with the Arabic and Zanzibar account of the fishing with the remora. We have here a transitional stage from the cormorant story to the *remora* story, as fathered by Columbus and permanently incorporated in all later accounts who drew upon the Columbus story.

[17] T. Domenichelli, "Sopra la vita e i viaggi del beato Odorico Da Pordenone," in Prato, 1881, p. 180 (cap. XLVI).

[18] *Ibid.*, p. 232.

[19] Yule, *op. cit.*, p. 352.

[20] *Ibid.*, p. 189.

Professor Gudger has shown, beyond any possibility of cavil, that all the accounts of the remora fishing in America recorded after Oviedo go back to this latter source, and I shall now show that Oviedo's account goes back, through Bernaldez, to an Arabic source, which is itself an evolution of the second Italian version of Odoric's cormorant fishing, as preserved to us in Ramusio.

Bernaldez[21] says: "For they call it hunting, and they hunt one fish with others of a particular kind," while in the "Journal of the Second Voyage"[22] we read: "The fishing consists in this that they take certain fishes which they call *revesos,* the largest of whom are not larger than pilchards," from which Peter Martyr made his "*reversus* fishes."[23] In the Spanish the passage in Bernaldez runs as follows:

> Vino una canoa a casa de pezes que ansí le llamaban ellos *caza,* que ***cazan con unos pezes otros.***

It will be observed that all the Columbus accounts tell of the invitation extended by the fishermen to Columbus to see the peculiar kind of fishing, and the giving of the catch to Columbus, according to Bernaldez, for a feast. This is identical with the manner in which Odoric tells of the invitation to watch the cormorant fishing. The resemblance is striking. Now, in the second Italian version in Ramusio the fish with which other fish are caught is called *marigione,* "diver," while others call it *sea-calf.* We have here, side by side, cormorant, otter and remora. I have already shown in my book, "Africa and the Discovery of America," that much of the matter in the "Voyages of Columbus" is apocryphal and comes from Odoric of Pordenone's "Itinerario," a name which Bernaldez uses for the book of Columbus, from which he got his information. There can be little doubt that the sec-

[21] *Loc. cit.,* p. 450.

[22] See my "Africa and the Discovery of America," Philadelphia, 1920, Vol. I, p. 64.

[23] Gudger, *loc. cit.,* p. 297.

ond Italian version was corrected or annotated by Columbus in the margin, where the true story of the remora fishing at Zanzibar was given from an Arabic source, from which Columbus retained two foreign terms. He had found in his source *kassa*, the turtle caught by the remora, and the name was apparently entered into the margin from which Bernaldez got his threefold *caza* "chase." Indeed, it appears that in his "que ansí *le* llamaban ellos *caza*," *it* referred originally to the fishes caught, that is, to the turtles, which from the resemblance to Spanish *caza*, "chase," produced the unfortunate pun. It will be noticed that in the "Journal of the Second Voyage" the corresponding passage runs "they take certain fishes which they call *reversos*," where the second Italian version says "fishing by aid of a certain fish called *marigione*," that is, "diver." Now the Arabic word for "diver" is *gavvāsah*. Anciently the initial guttural was rendered in Spanish by a simple *g*, but in the fifteenth century this Arabic word would sound to a European ear as *reverso* or *reveso*, which it actually assumed in the Columbus story. No such Spanish word is anywhere else recorded for the remora. Again, the marginal gloss, from Bernaldez, "hunting with a fish," must have been "caza con un pez," which Peter Martyr took to be the name of the fish, the remora, hence he misread the first as *guaicanum*, and called this the Indian name for the fish, a word which is only recorded as a quotation from Peter Martyr.

From the previous discussion it follows:

1. The remora fishing is very old and originated in the Indian Ocean, but did not get into literature before Columbus.

2. Odoric of Pordenone's cormorant fishing was from the start confused with the fishing by means of an otter and, in Ramusio's second version, was dimly identified with the remora fishing.

3. Ramusio's second version was, before the time of Columbus, influenced by an Arabic source or explained

by an Arab acquainted with the remora fishing at Zanzibar, and this new form supplied Columbus with the Zanzibar word for "turtle," namely, *kassa,* and the Arabic word for "diver," namely, the Spanish *reves* or *reverso,* which was wrongly attached to the "remora."

4. Bernaldez and Peter Martyr created a non-existing remora story for America out of Odoric's much-revised cormorant story, on the basis of some marginal notes in Columbus's "Itinerario," which itself was based on Odoric's "Itinerario," and referred to Zanzibar and not to America.

5. There are in America no corroborative stories of the remora fishing, except as derived from Oviedo's hearsay account, which itself is based on the accounts of Bernaldez and Peter Martyr, which, in their turn, are taken from a revised edition of Odoric's cormorant fishing story.

SHORTER ARTICLES AND DISCUSSION

REPORT OF THE COMMITTEE ON GENETIC FORM AND NOMENCLATURE

THE American Society of Naturalists at their meeting in 1919 appointed a Committee on Genetic Form and Nomenclature consisting of Drs. S. Wright, G. H. Shull, O. E. White, A. H. Sturtevant and myself as chairman. We were to consider the matter of genetic nomenclature and submit constructive suggestions for standardizing descriptive terms in this subject. "The following report of the committee was submitted to the meeting of the American Naturalists at Chicago, 1920, as a foundation intended to cover the cases of inheritance commonly met with by the majority of experimental workers in genetics. It is submitted in the hope that it may be published to invite discussion as to suggested modifications which would enable it to include particular problems of the scores of investigators in this field. In making such criticisms it is suggested that the primary object of this report be continually borne in mind and that constructive suggestions based on it as a framework are more likely to lead to beneficial results than purely destructive ones. The vast majority of workers in genetics will be concerned with simple enough problems to be covered by the report. Those whose material requires modification of the methods therein suggested will undoubtedly see the justice in attempting to adapt their particular needs to some modification of a system which will meet the needs of the majority."

C. C. LITTLE, *Chairman,*
Committee on Genetic Form and Nomenclature.

In submitting this report your committee desires to call attention to certain matters of general interest in connection with it. It is neither *proposed* nor *supposed* that those now familiar with some characteristic or individual form of genetic nomenclature will necessarily find it desirable to conform with the suggestions contained herein. If they can and will cheerfully do so, so much the better; if not, no intention to dictate is implied in this report.

It is, however, believed that a considerable number of geneticists will agree to the main suggestions of the report, and will thereby form a nucleus to which younger geneticists beginning publication would in a majority of cases join themselves. Thus, after a time, a far more uniform method of publication than now exists would become established.

In order to give such an opportunity, your committee respectfully suggests that this report, if approved by vote of the members present, be published at the earliest convenient time.

1. *The Type.*—In most animals and plants it is convenient to settle on a standard type, preferably the wild type, when this is known. The effects of the various genetic factors are in general to be measured by the departure from type which they bring about. This recommendation involves no real departure from the system now in use by most geneticists.

2. *Series of Allelomorphs.*—A single letter, with a subscript, *if necessary*, is to be assigned to each series of allelomorphs. This letter should, when possible, be chosen so as to give some hint as to the nature of the effects caused by variations in the series in question. The member of each allelomorph series present in the type is to be represented by the symbol for that series, capitalized and with no superscript. Factors dominant over the type are to be represented by the same capitalized symbol as the type, but with appropriate superscripts. Recessives are to be represented by the same symbol in lower case also with appropriate superscripts (*when necessary*). The symbols for the type factors may be omitted in formulæ where convenient. The agouti series in mice A^y, A^1, A, a^n, in which two factors are dominant over the wild gray type and one recessive is an example of the use of symbols. [This series might properly have been given a Y or B symbol in place of the A adopted. Since, however, it is to be thought of in terms of modification of the agouti pattern, the symbol A is chosen.]

3. *Dominance.*—Dominance of genes is recognized to be largely a matter of convenience. Factors may be considered dominant which produce an easily recognized departure from type, when heterozygous.

4. *Superscripts.*—It is suggested that both a literal and a numerical superscript be assigned, *upon the initial description,* to each factor except the type (at least in series of multiple allelomorphs). EITHER SUPERSCRIPT MAY THEREAFTER BE USED ALONE. The numerical superscript shall indicate the estimated

degree of divergence from type, produced by the factor in question in a scale in which 10 is the apparent physiological or visible limit and 0 is the type. Thus A^{10} and a^{10} represent factors which cause deviations to the physiological limit in opposite directions (self yellow and self black) from the type A (agouti). A^4 (light-bellied agouti) represents an estimated deviation between ticked bellied agouti (A) and yellow (A^{10}). *The order of effect is more important than a precise estimate of the degree of effect.* Decimals and numbers beyond 10 may be used whenever necessary, in event of grades not believed physiologically possible. A superscript, once adopted, should not be changed, which also applies to all other symbols. The value of making provision for a system to *indicate the order* of a multiple allelomorph series is clear; the numerical symbols will only be *used* when such a situation is encountered.

5. *Independent Factors.*—Independent series of allelomorphs should be represented by different letters or, if desired, by the same letter with different following numbers. Symbols composed of two or more letters should not be used. It is suggested that factors with more or less similar effects be represented by the same letter with different following numbers, as S1, S2, S3, etc. The same symbol may conveniently be used for factors with more or less similar effects in different animals and plants without implying identity.

6. *Doubtful Factors.*—In case the formula of an individual is not fully known, the uncertain factor may, if desired, be represented by a superscript X (or ?) or the whole symbol may be replaced by a dash. Thus C^x C^x (or $C^?$ $C^?$) means *complete* ignorance of the factor in series C. CC^x, $CC^?$, or C — represents ignorance as to one of the factors in the zygote. If there is partial knowledge, a double (or triple) superscript may be used to indicate the various possibilities. Thus the progeny of the cross $CC \times c^r c^a$ may be represented by $C\ c^{ra}$, a form which gives more information than C —.

7. *Modifiers.*—The symbol [ ] containing appropriate symbols represents residual heredity of the kind indicated. Thus [S +] is a convenient method of representative + modifiers of the effect produced by the S (Spotting) series of allelomorphs. In a detailed study of a particular group of modifiers, the parenthesis may well contain the grade of effect produced by the modifiers in the case in question. Thus [+ 4.2] and [—2.5] might be used to represent the modifiers of typical hooded rats of grades

+4.2 and −2.5. The modifiers of a cross bred may be indicated in some appropriate manner as [+4.2, −2.5].

8. *Linkage* is best represented by the fractional form used by workers on *Drosophila*. The factors are written in the order of linkage, omitting type factors.

COMMITTEE ON GENETIC FORM AND NOMENCLATURE

STANDARDIZED MICROPHOTOGRAPHY

SECOND CONTRIBUTION: THE OBJECT FACTOR

IN my first contribution to the subject of standardized microphotography, published in the *Anatomical Record*, I have pointed out the variables and the methods which I have pursued in treating them. One, or perhaps more correctly a group of variables were, however, left out of consideration quite purposely because of the difficulty in finding for them a standard of permanent value. I have in mind the microscopical section itself or what may be properly called the object factor. The following four elements enter into its composition: (1) the thickness of the section, (2) the light absorption coefficient of the tissue, (3) the relative luminosity of the different stains and (4) the depth or intensity of staining. The second and third of these component elements may be disregarded since experience shows us that exposure is very little influenced by them. There remain, however, the first and fourth, and to determine the influence of these on exposure the following experiments were undertaken.

First of all, to avoid all possible error, slides were chosen of uniform thickness measured with a Ciceri Smiths Patent Micrometer so as to be sure that the distance of the section from the substage condenser should in every case be the same. The cover-glasses were also of uniform thickness. The stomach of a frog preserved in Zenker's fixing fluid was sectioned into series of 5, 10 and 20 micromillimeter thick sections on a Minot microtome and care was taken to have in each case a ribbon of 100 even sections, thus more or less assuring their uniform thickness. The sections were stretched on distilled water heated over a flame and no cement of any kind was used. On one slide three sections of each thickness were placed. On other slides sections only of one kind were placed and their thickness marked in every case by a carborundum pencil.

The slide with all three kinds of sections was stained for 12 hours in alumcarmine, a stain which, as is well known, does not overstain. They were then washed in water and again stained for 12 hours in a weak alcoholic solution of Bleu de Lyon. All sections on this slide received therefore the same treatment and the difference in the depth of stain was entirely due to the thickness of the section stained.

The other slides were treated in a different way. They were stained in Delafield's hæmatoxylin followed by tetrabromfluorescic acid. This stain was chosen because it is possible at will to control the depth of staining. A set A of three slides, one with 5 micromillimeter sections, one with 10 and one with 20, was treated simultaneously in a Coplin's staining jar. The sections were therefore stained, washed, destained in acid alcohol, treated with ammonia alcohol, stained in a weak solution of tetrabromfluorescic acid in 95 per cent. alcohol and washed in pure alcohol the same length of time. In regard to depth of stain these slides presented, therefore, the same conditions as the slide stained in alumcarmine and Bleu de Lyon.

Several other slides were treated individually in the same stains. They were all first considerably overstained in Delafield's hæmatoxylin, washed in water and destained in acid alcohol until they had when viewed over a white surface, the same shade of color to the naked eye, regardless of the thickness of the section. If after treatment with ammonia alcohol the blue color was not approximately of the same shade, the darker slide was again transferred to the acid alcohol, until all sections looked approximately alike. They were now stained in the tetrabromfluorescic acid, the thickest sections remaining a short time in the fluid, the 10 micromillimeter sections somewhat longer and the 5 micromillimeter sections longest, and again compared over a white surface. From several slides thus prepared three were selected which to the naked eye were to all purposes of the same appearance, although their respective thickness were 5, 10 and 20 micromillimeters. Here then we had a Set B of sections in which the depth of stain had nothing to do with the thickness of the section, but was entirely dependent upon the amount of stain absorbed by the tissues.

Fractional exposures on orthonon plates with Cramer ray-filters were made on our standardized microphotographic apparatus and developed by the time and temperature method with

fresh developer for each plate. In the case of the first slide stained in alumcarmine and Bleu de Lyon as in the Set A, the normal exposure for the 10 micromillimeter section was twice that for the 5 one, and the normal exposure for the 20 micromillimeter section was four times that of the 5 one. In the case of the Set B all slides required the same exposure.

In analyzing the results thus obtained we come to the conclusion that the thickness of the section, within the limits given, has no influence whatsoever on the length of exposure; but that the latter stands in a direct ratio to the amount of stain absorbed by the tissue.

For practical purposes, especially for those who are using my table of R-P factors, the results of this investigation may be interpreted as follows: disregard the thickness of the sections and the appearance of the stain under microscope and pay attention only to the intensity as it appears to the naked eye. The table refers to normally well stained sections of medium thickness. Double the exposure for darker appearing and reduce the exposure by half for lighter appearing sections.

It will be observed that in the experiments elements 2 and 3 remained of constant value for the simple reason that the tissue used was not only the same in kind, but actually from the same piece of organ and was stained in the same stain in each set of slides. A comparison of the length of normal exposure in the case of sections stained in alumcarmine and Bleu de Lyon with those stained in hæmatoxylin and tetrabromfluorescic acid serves to confirm my statement at the beginning of this article, that the relative luminosity of different stains may be entirely disregarded in the matter of exposure.

ALEXANDER PETRUNKEVITCH

YALE UNIVERSITY.

SEX RATIOS IN PLATYGASTER

IN the *Journal of Heredity* for last year (Vol. X, p. 344) I published data on sex ratios in three species of polyembryonic Hymenoptera. One of these is *Platygaster felti*, which parasitizes the eggs of two species of the Cecidomyiidæ, *Walshomyia taxana* and *Rhopalomyia sabinæ*. These flies make their galls on the mountain cedar, *Sabina sabinodes*.

Since the above data were published I have had an opportunity to breed out in the laboratory a total of 200 broods of

Platygaster; 73 from the carcasses of *Rhopalomyia* and 128 from those of *Walshomyia.* The remarkable character of the sex ratios, as revealed in the published data, is emphasized by the additional facts secured from the more recent rearings. It therefore seems worth while to publish the full data, which is given in condensed form in the following table. In the first column the total number of individuals in each brood is given; in the second, the number of females; and in the third, the number of males. In the fourth column are listed the number of broods showing the combination of females and males in the corresponding horizontal line.

The total number of individuals in the 200 broods is 2,722, of which 2,346 are females and 376 males. The average per brood is 13.61. The size of the brood reared from *Walshomyia* is very much smaller than those from *Rhopalomyia.* There are 1,417 individuals in the 128 broods from *Walshomyia,* or an average of 11.07 per brood. The broods from *Rhopalomyia* have 1,305 individuals, or an average of 18.12 per brood. This represents an average increase of 63.6 per cent. The rate of increase in the number of males per brood from *Walshomyia* to *Rhopalomyia* is almost the same as this. The average number of males in broods from *Walshomyia* is 1.55, and in those from *Rhopalomyia* is 2.45. This represents an increase of 58 per cent.

The difference in the size of broods is, in all probability, due to the difference in the size of the two host larvæ. The larva of *Rhopalomyia* is almost twice as large as that of *Walshomyia,* and hence must furnish a more abundant food supply for the multiplication of embryos at the time of their formation in the polygerm.

One of the most striking facts in the data is the preponderances of females. Approximately 86 per cent. of the individuals are females. No male brood has been found, and in not a single instance does the number of males exceed or even equal the number of females in a brood. There are, however, nine pure female broods. Of the 191 mixed broods 113, or 59.61 per cent., have a single male present in each brood. The other 78 broods show the following numbers of males: Thirty with two males each; twenty-four with three each; ten with four each; four with five each; four with six each; three with seven each; two with eight each; and one with ten.

Another interesting fact is the frequent occurrence of broods with a single male. Almost sixty per cent. of the mixed broods

TABLE I

BROODS OF PLATYGASTER FELTI

No. of Individuals	Females	Males	No. of Broods
5	3	2	1
5	4	1	1
6	5	1	4
7	5	2	1
7	6	1	14
8	6	2	2
8	7	1	9
9	7	2	2
9	8	1	13
9	9	0	3
10	7	3	1
10	8	2	1
10	9	1	31
10	10	0	1
11	8	3	2
11	9	2	4
11	10	1	15
12	10	2	2
12	11	1	3
13	9	4	1
13	10	3	3
13	11	2	3
13	12	1	5
13	13	0	1
14	10	4	1
14	11	3	2
14	12	2	2
14	14	0	1
15	12	3	1
15	13	2	6
15	14	1	7
15	15	0	1
16	13	3	2
16	14	2	2
16	15	1	3
16	16	0	1
17	13	4	1
17	14	3	2
17	15	2	2
17	16	1	2
18	14	4	1
18	15	3	2
18	17	1	4
19	15	4	1
19	16	3	3
19	17	2	1
19	19	0	1
20	16	4	1
20	17	3	1
21	17	4	1
21	18	3	1
22	17	5	2
22	20	2	1
22	21	1	2
23	19	4	1
23	20	3	2
24	20	4	1
25	17	8	1

TABLE I (*Continued*)

BROODS OF PLATYGASTER FELTI

No. of Individuals	Females	Males	No. of Broods
26	20	6	1
26	21	5	1
26	22	4	1
26	23	3	1
27	21	6	1
28	25	3	1
29	24	5	1
31	24	7	2
34	26	8	1
34	27	7	1
36	26	10	1
36	30	6	1
37	31	6	1

are of this character. Certain combinations, such as nine females and one male, occur with very great frequency, suggesting that a single male is produced at some definite point in development. Statistical treatment of the data supports this suggestion. My colleague, Dr. Muller, has kindly calculated the standard error and standard deviation of the numbers of males per brood, and finds that they are 1.17 and .64 respectively. These results show that the number of males produced per brood does not vary as much as it would if males were formed at random. If males were produced at random, the amount of variation in the number of males would be expressed by a standard deviation of 1.17. This means that there is a tendency to have the production of males confined to particular cells in the embryonic mass, so that only one or two males are usually formed in a brood.

The fact that the parasite deposits one egg at each oviposition makes it practically certain that the mixed brood of *Platygaster* is the product of a single fertilized egg. The important question is how the single male originates during the course of development. I have elsewhere discussed this question, and have suggested that the appearance of one or more males in a brood may be due to an abnormal behavior of the sex chromosomes. An abnormal division causing the loss of an x-chromosome from one of the early blastomeres would explain the appearance of a mixed brood, for such a cell could become the progenitor of one or more males.

J. T. PATTERSON

UNIVERSITY OF TEXAS

REPRODUCING POWER OF WELL-FILLED VS. POORLY-FILLED EARS OF MAIZE

THESE tests were conducted to determine the effects, if any, on the progeny, particularly as to productivity, when a stalk of corn is caused to produce comparatively few kernels instead of a normal-sized, well-filled ear. In other words, the object was to learn whether artificially reducing the possible number of progeny kernels would have any influence on their viability, vigor or ability to yield.

In selecting seed corn, ears are occasionally found which evidently would have been much larger and better filled had not something such as an overhanging blade or an insect interfered with pollination. Are such ears suitable for seed?

Similar tests were conducted with three varieties, one being a cross-bred variety. The three classes of seed for these tests were grown in 1914 and their comparative productiveness tested in 1915. The seed of U. S. Selection 77 was grown and tested at Piketon on river-bottom soil in Southern Ohio, and that of the other two varieties at Broad Run on Piedmont clay of Northern Virginia.

METHODS OF PROCEDURE

Two methods were used to control the pollination and consequent seed production of the poorly filled ears. In one case, the first silks to appear were about an inch beyond the end of the shoots when the shoots were bagged to prevent further pollination. In the other case, the ear shoots were bagged before the silks began to appear. When all the silks had protruded several inches the bags were removed for half an hour and then replaced. This was done when pollen was falling freely. A few of the uncovered silks thus became naturally pollinated.

The first method produced ears the butt ends of which were fairly well filled for about one fourth the length of the cob. The second method gave ears that had a few large rounded kernels scattered over the cob. As check seed for the tests, large, well-filled, typical, seed ears that had been allowed to mature unmolested were selected. The seed ears of the three lots of each variety were selected from the same rows from similar stalks grown under like conditions as far as possible. The drying, care, etc., were the same for each of the three lots.

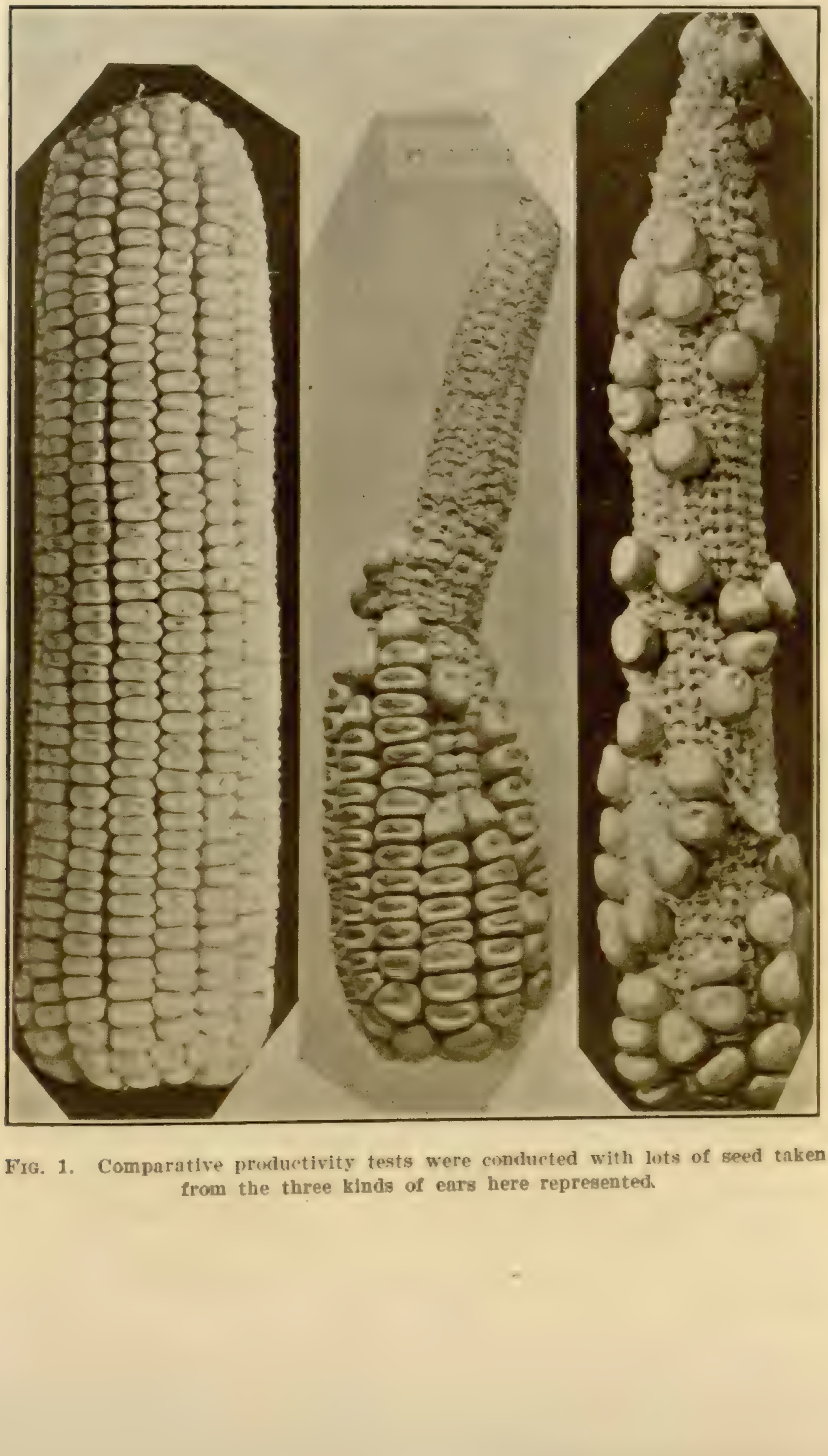

FIG. 1. Comparative productivity tests were conducted with lots of seed taken from the three kinds of ears here represented.

The seed from the check ears will be referred to as such while that from the ears filled only at the butt ends, as Lot 2, and that from the ears with kernels scattered over the cob as Lot 3.

The number of ears used in making up each lot of seed follows: Selection 77, Check, 62 ears; Lot 2, 67 ears; Lot 3, 54 ears; Selection 119, Check, 26 ears; Lot 2, 19 ears; Lot 3, 26 ears; and Cross 182, Check, 26 ears; Lot 2, 14 ears; and Lot 3, 19 ears.

In preparing for planting each lot was composited in the following manner. The same number of kernels was taken from each ear of a lot and these kernels combined made just enough to plant one row 50 hills long. The comparative weights of the three lots of seed are given in Table I.

TABLE I

WEIGHTS IN GRAMS OF 265 KERNELS OF EACH LOT OF SEED

Variety	Check Seed	Lot 2	Lot 3
Selection 77	124	141	137
Selection 119	118	122	127
Cross 182	126	148	151

DATE AND METHOD OF PLANTING

U. S. Selection 77 was planted May 1, 1915. The three lots were planted in adjacent rows and the test repeated 14 times. Selection 119 and Cross 182 were planted May 3, 1915. The lots of these two varieties were planted in the same order as the lots of Selection 77, but only 15 rows each of these two varieties were planted. The planting was done by hand and later all the rows were thinned to a uniform stand.

OBSERVATIONS DURING GROWTH

At no stage during the growth of the corn was there any noticeable difference among the three lots. Neither was there any difference in the time of silking and tasseling, in the height of stalk, nor in time of maturing. There was a slight difference in the field germination of the three lots of seed as shown in Table II., but these differences within the varieties are not great enough to be significant nor are they consistent for the three varieties.

TABLE II

Variety	Lot	Field Germination, Per Cent.	Ave. Weight of the Ears Produced, Pounds	Total No. of Stalks	Total Yield of Ears, Pounds	Corrected Yield per Acre, Bushels
Selection 77.....	Check	87.6	0.763	1,725	1,292.0	96.7
	2	86.6	0.736	1,738	1,228.0	89.9
	3	86.6	0.761	1,723	1,277.0	94.5
Selection 119....	Check	84.5	0.592	481	308.1	74.8
	2	84.5	0.586	472	283.1	70.9
	3	79.7	0.585	494	290.6	69.7
Cross 182.....	Check	83.8	0.682	503	346.4	80.1
	2	88.3	0.635	507	321.2	74.8
	3	80.8	0.662	510	331.4	77.0

Results

The yields in Table II. are all based on field weights at harvest time. With all three varieties the well-filled seed ears produced the highest yields, the increase being from 2.2 to 3.9 bushels per acre over the next highest lot. In the 25 comparisons between the two lots, Checks outyielded Lot 3, its nearest competitor, 16 times, with one tie; and it outyielded Lots 2, 20 times with two ties.

Previous work had proved Cross 182 more productive than Selection 119. In the tests reported in Table II. they occupied the same amount and kind of soil and Cross 182 is consistently more productive than Selection 119.

Regarding all three varieties the ears harvested from each of the three lots of seed were equally well-filled and of the same general appearance. These tests warrant the conclusions that ears poorly-filled by reason of withheld pollen will not transmit this character to their progeny, and can be expected to supply seed almost as productive, if not as productive, as they would have supplied if completely pollinated.

C. P. Hartley,
H. S. Garrison

U. S. Department of Agriculture

ANTHEROPHAGUS OCHRACEUS MELS. IN THE NESTS OF BUMBLEBEES

The recent appearance of two articles on "The Phoresy of *Antherophagus*," one by W. M. Wheeler (1919) and the other by H. Donisthorpe (1920) have prompted me to publish some additional observations on the habits of *Antherophagus ochraceus* Mels. in this country.

Wheeler writing in December, 1919, recorded the capture of an adult of this beetle near Colebrook, Connecticut, attached to the proboscis of a worker bumblebee (*B. vagans*). When the bumblebee, which vainly tried to rid herself of the beetle, was placed in a cyanide jar, the beetle still maintained its hold. In this same article there is a discussion of the phenomenon of "phoresy" as defined by Lesne (1896) and expanded by Janet (1897), together with an account of the known habits of *Antherophagus* and a bibliography. Donisthorpe in October, 1920, published a résumé of Wheeler's paper, presenting further information concerning "phoresy" in general, the habits of *Antherophagus*, and additional references. Scott (1920) has also contributed to our knowledge of the biology of these beetles.

Many of the rather numerous European references report the finding of *Antherophagus* (*pallens*, *silaceus* and *nigricornis*) and of *Cryptophagus* (*setulosus*, and *sp.*) in the nests of various species of bumblebees. The only American record actually citing an instance of finding *Antherophagus ochraceus* Mels. in the nests of bumblebees is that given by A. S. Packard (1864) based on observations made by F. W. Putnam in Massachusetts and Vermont. J. B. Smith (1909), without giving any data, says that *Antherophagus* occurs in the nests of bumblebees. This latter note is probably based on the statements by Packard, or else on accounts of the habits of the European members of this genus.

While in Wisconsin last summer (1920), I was able to examine many bumblebees nests in various parts of that state, through the kindness of Dr. S. B. Fracker. On two different occasions I found *Antherophagus ochraceus* (C. A. Frost det.) in the nests. In one nest of *Bremus* (*Bombus*) *fervidus* (Fabr.) examined August 12, 1920, were eighteen adult specimens of this beetle.

At the same time and place I took thirty-four larvæ of a small beetle in various stages of development. As these larvæ were associated with the beetles and as they agree with the figure and brief description of *A. ochraceus* given by Packard (1883), I assume them to be the same. In another nest of *Bremus* (*Bombias*) *auricomus* (Robt.) opened on July 26, 1920, at Clyman Junction, Wisconsin, I found a single adult of *A. ochraceus.* Again on October 3, 1920, near White Heath, Illinois, I collected about a dozen small beetle larvæ in a surface nest of *B. Pennsylvanicus* (DeGeer). These last-mentioned larvæ differ slightly from those found in the nest at Baraboo, Wisconsin, and if not the same species may represent another species of *Antherophagus.*

There has been much discussion as to the feeding habits of the adult and larval *Antherophagus.* Wheeler, after a survey of the literature of the subject, came to the conclusion that the larvæ were "in all probability merely scavengers in the *Bombus* nests." Wagner (1907) expresses the idea that they "will occasion enormous destruction in the nest," but without giving an instance of the same. I believe that these insects are purely scavengers, not only feeding on the excrement of the bumblebees as suggested by some, but also on all kinds of refuse as maintained by Reuter (1913). In the nests I examined containing *Antherophagus ochraceus,* the beetles and larvæ were never on that portion of the comb then being used by the bees. They were always either on the old decaying empty cocoons on the bottom of the nest, or in the débris directly beneath or surrounding the comb. Such are not the habits of the true parasites and harmful inquilines of bumblebees. The larvæ of *Vitula* (*Nephopteryx* in litt.) *edmansii* described by Packard from the nests of bumblebees feed on the pollen, honey, wax or cells of the comb. To escape being killed by the bumblebees or carried out of the nest, the larvæ of this moth spin a regular labyrinth of silken tubes or cases and never expose themselves to the bumblebees. The larvæ of *Antherophagus* do not spin protective cases and are in no sense of the word parasitic on the adult bees, larvæ or pupæ. If they, thus unprotected, should crawl conspicuously over the comb to destroy the eggs, larvæ or pupæ, or to eat the new comb and stored food, they could easily be combated by the bumblebees. Furthermore, the nest containing the thirty-four larvæ and eighteen adult beetles taken at Baraboo, Wisconsin, showed no signs of the great destruction mentioned by Wagner. For that time of

year, August 12, it was in fact a strong colony, containing ninety-one workers, fifty-six pupal cocoons, and large stores of honey and pollen. It is possible that the upper part of the comb of a bumblebee nest might develop so swiftly in some cases, as to cause some cells either filled or not filled with pollen and honey on the lower part of the comb to be neglected, and thus infested with *Antherophagus*. This last statement, however, would certainly be the exception rather than the rule. In the cases that have come under my observation *A. ochraceus* played the rôle of a scavenger, in the débris beneath and about the nest, feeding on the refuse comb, feces, honey, or bits of pollen and wax that perchance had fallen to the bottom of the nest.

Wheeler voices the opinion of Sharp (1899) that the instincts of the beetle permit it to recognize the bumblebee, but not to enable it to find the nest. Therefore the beetle waits on flowers until it can attach itself to a bumblebee and be conveyed to the nest of the latter. Donisthorpe suggests that "it is not so much that they [*Antherophagus*] lack the instinct to find the bee's nest, but rather that it gives them protection from their hosts when they arrive there." *Antherophagus* may or may not be able to find the nests of bumblebees of its own accord, but I am inclined to doubt whether the occasion of the "phoresy" is protective, in that it gives "them protection from their hosts when they arrive there," by their having acquired the nest "aura." If *Antherophagus* is a scavenger, as the evidence seems to indicate, and keeps well hidden in the débris on the bottom or sides of the comb, why is there a need for a nest "aura"? One of these beetles carried to a bumblebee's nest, in all probability, soon after arriving there, releases its hold and falls down to the lower part or bottom of the nest. Many other beetles are accidental visitors or inhabitants of such nests, and living thus in the material about and beneath the comb are not noticed by the ever-watchful bees and go unmolested. W. H. Tuck (1896, 1897) lists over sixty species of beetles from the nests of various species of bumblebees in England, most of which are undoubtedly only casual intruders. I have taken specimens of *Harpalus* sp. and *Onthophagus hecate* Panz. in the nests of bumblebees. Such beetles are much larger than *Antherophagus*, are not even considered as "anthophilous" (Lovell, 1915), nor have they ever been accredited with habits of "phoresy." Evidently then, such beetles gain entrance to the nest and live there for a time at

least without having first acquired a nest "aura." I believe that *Antherophagus* often, if not always, forces the bee to carry it simply in order to find the nest, and not to acquire a nest "aura," such as all the bees of each and every colony possess. If *Antherophagus* had habits similar to those of the inquiline-bee *Psithyrus*, there would be an advantage in having a nest "aura."

Scott (1920) says that

> Presumably these [*A. pallens*] beetles are double-brooded, with a short summer generation intervening between the emergence of the adults in May and the assumption of the resting condition by the larvæ in autumn.

I have taken adults of *A. ochraceus* by sweeping flowers on May 7 and 23, 1917. The insect collections of the Illinois State Natural History Survey contain adults taken on July 19, 23 and 30, 1891, and August 15, 1893. Blatchley (1910) mentions the species as occurring on flowers, June 24 to September 21. As previously mentioned I took one adult in a bumblebee nest on July 26 and eighteen more on August 12. The adults taken on May 7 and 23 certainly represent the hibernating brood. Those found both out of doors and also in a nest on July 19–30, are in all probability the adults of the first brood or summer generation. Those taken on August 12–15, may represent a true second summer generation, but more than likely belong to the same brood of July 19–30. Scott found that *A. pallens* hibernated as larvæ, pupating in early summer. Some of the larvæ presumably those of *Antherophagus* which I took on October 3, when examined on November 11, 1920, had constructed cells in the earth on the bottom of the rearing jar; thus indicating that they hibernated as larvæ. Summarizing these records: *A. ochraceus* is probably double-brooded, hibernating as larvæ in cells in the soil or material about or under the bumblebee nest.

THEODORE H. FRISON

UNIVERSITY OF ILLINOIS

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THE INTERNAL SECRETIONS IN GROWTH AND DEVELOPMENT OF AMPHIBIANS

EDUARD UHLENHUTH, PH.D.

(From the Laboratories of the Rockefeller Institute for Medical Research, N. Y.)

WHILE up to 1910 the higher vertebrates were used predominantly in the study of the internal secretions, during the last decade the larvæ of the amphibians have been found an excellent material, suitable for the investigation of many problems of endocrinology. To-day the results obtained in this work seem to form a solid mass of trustworthy evidence, from which may be derived not only valuable information as to the mechanism of growth and development in amphibians, but also important knowledge as to the functions of certain endocrine glands. In the field of internal secretion, these experiments have attracted increasing interest from the beginning. It is evident at present that further clarification of many of the more important problems of internal secretion will come from the work on amphibians, as it can be and has been carried on with methods far superior to those available in the work on higher vertebrates.

Before entering into details the most prominent facts as revealed in the amphibian experiments may be pointed out. In the control of growth and development of the amphibian organism, the thyroid and pituitary glands play the most important rôles. The thymus is not concerned with the growth and development of amphibian larvæ. The functions of the thyroid and hypophysis glands, as far as they are revealed in the processes of

growth and development, exhibit a remarkable resemblance, and the secretions of these two glands can replace each other to some degree, but for the most part are specific.

Among those who have worked out these facts, J. F. Gudernatsch, Leo Adler, Bennet M. Allen and his pupil W. W. Swingle, E. R. Hoskins and M. M. Hoskins, and P. E. Smith deserve the greatest credit. Since much of their success is due to the extirpation of the endocrine glands in early embryonic stages, we should mention here also the names of three investigators, namely Gustav Born, Herman Braus and Ross G. Harrison, who have elaborated the delicate technique employed in the extirpation experiments and thus have made possible the progress which has been derived from them.

We will begin with the thyroid mechanism, as it has been studied more thoroughly than other glands, and in fact seems to be the chief factor in the control of growth and development. Its study in the amphibians as well as the entire work on amphibians was initiated by the well-known experiments on thyroid-feeding to tadpoles as carried out by Gudernatsch (1).

If tadpoles are fed fresh thyroid gland or are kept in water to which minute amounts of thyroid extract are added, a remarkable acceleration of development takes place. This development is the more conspicuous as it may occur with complete absence of growth. In tadpoles it is characterized especially by the sudden development of the fore limbs, by the atrophy of the tail, a sudden protrusion of the eye-balls (2), by the rapid shortening of the spiral gut, by the precocious atrophy of the organs of the larval mouth, which are replaced by the frog mouth (3), and by precocious ossification (4). These experiments have been repeated with the larvæ of salamanders, in which the precocious occurrences of the first moult, the atrophy of the gills and absorption of the fin of the tail, are most conspicuous effects of the thyroid application (5).

The rapidity with which these processes may take place is one of the most remarkable features of the thyroid effect. Two normal larvæ of the species *A. opacum*, for instance, metamorphosed at an age of 86 days and measured 60 mm. at this time. Six other larvæ of the same brood were placed in an emulsion of iodothyrine at an age of 35 days, at which time they measured 30 mm. on the average. One week later, at an age of only 42 to 43 days and a size of 24 mm., all had metamorphosed. Moreover, 5 days after metamorphosis, *i.e.*, at an age of 47 days in one animal which was examined in sections, the visceral skeleton had undergone all those complicated changes through which the gill arches of the larvæ develop into the hyoid apparatus of the adult. The effect of the thyroid hormone is quantitative; the acceleration of the amphibian metamorphosis increases with increasing concentration of the thyroid emulsion, as shown in Table I.

TABLE I

QUANTITATIVE EFFECT OF THE THYROID HORMONE IN THE ACCELERATION OF THE METAMORPHOSIS OF *Ambystoma maculatum*

Quantity of Iodothyrine	Age at Metamorphosis		
	Control	Iodothyrine	Difference
0.1 gm. iodothyrine in 1,000 c.c. of water	101 days	33 days	67 per cent.
0.01 gm. iodothyrine in 1,000 c.c. of water	80 "	58 "	28 " "

In one experiment the larvæ of *Ambystoma maculatum* were kept in water, to which 0.1 gm. of iodothyrine per 1,000 c.c. of water had been added; in another experiment only 0.01 gm. of iodothyrine was added to 1,000 c.c. of water. In the first experiment all larvæ metamorphosed 13 days after the first application of iodothyrine; in the second experiment metamorphosis took place 39 days (on the average) after the first application of iodothyrine. The difference between the normal time of metamorphosis and the time of metamorphosis of the experimental larvæ was 67 per cent. in the first experiment and 28 per cent. in the second.

It is remarkable that the administration of the same amount of iodothyrine causes metamorphosis of salamander larvæ of different species in nearly the same interval of time. Thus, 0.1 gm. iodothyrine per 1,000 c.c. of water caused metamorphosis of *A. opacum* larvæ in 7 days, of *A. maculatum* larvæ in 13 days and of *A. tigrinum* larvæ in 13 days. The time required to induce metamorphosis in thyroid-fed tadpoles decreases with increasing age of the tadpoles. Gudernatsch (1) found that thyroid feeding caused metamorphosis in 20 days if tadpoles of a certain age were employed, in 6 days, if tadpoles 7 days older than the first lot were employed, and in only 4 days if the tadpoles were 14 days older than the first lot.

As pointed out above, the larvæ, when fed thyroid substance, may undergo the most remarkable development, although no growth may take place. This seems to be of great importance in many ways. In all organisms development and growth, under normal conditions, proceed in a parallel way. The behavior of the thyroid-fed larvæ suggests that the reason why no development takes place without growth is the fact that, under normal circumstances, the substances which cause development of certain organs are supplied through the same reactions which control the growth of the organism. If these substances are supplied to the organism from without, development may proceed at a higher rate than growth or may proceed even in the complete absence of growth and thus the relation between growth and development may become changed as in the thyroid-fed larvæ.

The changes of the relation between growth and development furnish an important link in the chain of facts that we must know in order to understand the mechanism of the thyroid apparatus as well as that of the amphibian metamorphosis. Although under certain conditions growth may be inhibited completely upon the feeding of thyroid, this is not always the case. Both the rate of development and the rate of growth are dependent

on the quantity of thyroid substance administered to the larvæ. Up to a certain quantity, growth as well as development is accelerated; if the quantity administered is further increased, growth becomes more and more inhibited, while differentiation is increasingly accelerated. With very large doses the thyroid substance may effect even a decrease in the size and weight of the larvæ; while development of the limbs is greatly accelerated in the beginning, it finally stops and the animals die from emaciation (6).

Kendall (7) has shown that in man the thyroid hormone increases the basal metabolism in a strictly quantitative way. Determinations of metabolism have not been made in amphibians, but the behavior of the thyroid-fed tadpoles as described above indicates that the thyroid hormone also increases highly the metabolism of the cold-blooded organism. If too much of the hormone is administred, metabolism is increased in such a manner that catabolism becomes higher than anabolism, since the organism no longer is capable of supplying enough food materials from outside to maintain a positive metabolic balance, and consequently the body substance itself is broken down and a decrease in size and body weight takes place. Finally even development becomes impossible. For this reason, as Lenhart (6) showed, more thyroid substance can be administered without leading to a check of development if the thyroid-fed larvæ are either kept under conditions which decrease metabolism, *i.e.*, in low temperature, or are fed on substances (carbohydrates) which can be made easily available for metabolism.

From these facts it seems evident that the amphibian metamorphosis is the result of a highly increased metabolism, or more correctly, metamorphosis seems to result if metabolism is increased in such a degree and manner that catabolism becomes higher than anabolism. The question arises whether substances or agents other than

thyroid substance can cause such an increase of metabolism as to bring about metamorphosis. Several experiments have been carried out to answer this question. But until thyroidectomized tadpoles have been employed in these experiments, no definite conclusions are possible; in larvæ possessing a normal thyroid gland it can not be decided whether the experimental conditions employed have caused metamorphosis by raising the metabolism directly or merely through the intermediation of the thyroid by precociously releasing the thyroid hormone. Powers as well as Barfurth has shown that a sudden cessation of food supply results in precocious metamorphosis. Although this is certainly true, it does not decide the point in question, but may mean that sudden starvation may precociously release the thyroid hormone. At any rate, starvation in itself does not cause metamorphosis, but is effective only if well-fed larvæ which are approaching metamorphosis and possess a thyroid capable already of functioning are suddenly starved. The same criticism applies to Kaufman's (8) recent experiments, in which an advanced neotenous larva (axolotl) of *Ambystoma tigrinum* was given salicylic acid, whereupon it metamorphosed promptly. This result is extremely interesting as it raises most urgently the question whether the action of iodothyrine is specific and whether the changes of metabolism resulting from thyroid administration are merely quantitative or also qualitative. As pointed out, Kaufman's experiment, however, does not answer any of these questions.

In accord with the highly increased catabolism as caused by the action of the thyroid hormone is the fact that metamorphosis, in its initial stages, appears to be more a process of profound atrophy than one of constructive development, although phenomena of the latter kind frequently accompany the degenerative processes. Among the most conspicuous processes of destruction are the complete atrophy of the gills and the entire vascular apparatus which serves the gill circulation, a con-

siderable destruction of the cartilaginous visceral skeleton, the atrophy of the larval mouth in anurans, the reduction of the intestinal coils in anurans, the complete atrophy of the tail in anurans and the atrophy of the fin in urodelans. Not before this extensive breakdown of the larval tissues has taken place and out of the remnants of the destroyed organs the new organs of the adult develop. Particularly instructive in this regard is the development of the epithelial bodies in the larvæ of salamanders; these develop from the epithelium of the destroyed gills and in the midst of the masses of detritus which result from the destruction especially of the gill vessels.

The fact that metamorphosis can be brought about by feeding mammalian thyroid substance to the amphibian larvæ, does not of course prove that the amphibian metamorphosis, under normal circumstances, is the result of the function of the amphibian thyroid gland itself. This, however, is the case, as demonstrated especially by the work of Allen (9) and of E. R. and M. M. Hoskins (10). If in an early embryonic stage of the anuran organism the thyroid is extirpated, metamorphosis can not take place at all and the tadpoles remain permanently (as far as the observations go) in the stage of an aquatic amphibian larva. Growth likewise is ultimately interfered with, although the thyroidectomized tadpoles may grow more rapidly in the beginning and even grow larger than normal tadpoles. On the other hand, if the thyroid of metamorphic tadpoles is grafted to tadpoles which are in early larval stages, metamorphosis of the latter, up to the stage of the larvæ from which the thyroid graft was taken, is caused (11). The metamorphosis of the amphibian eye is likewise impossible if it is removed from the influence of the thyroid hormone which controls the development of the eye. If eyes of old salamander larvæ are grafted to young larvæ, the metamorphosis of the graft may be retarded by as many as 7 months and will

not take place before the eyes of the host metamorphose. On the other hand, eyes of young larvæ, if they are grafted to old larvæ, can be made to metamorphose earlier than they would under normal conditions (12).

It has been said that the thyroid substance does not actually *produce* new characters, but merely accelerates the rate of their development which is predetermined by heredity. There can be little doubt, however, that the advance of the higher vertebrates from an aquatic stage, with open gill slits and internal or external gills, and in particular all the characters distinguishing the terrestrial amphibian from the aquatic larva, could not have developed if the thyroid apparatus had not attained, at some evolutionary stage of the amphibians, its present function. For the benefit of those who might think that the relatively short time (about 1½ years) of observation in Allen's and Hoskins's experiments does not justify this statement, I may refer to the Texan cave salamander, *Typhlomolge rathbuni* which illustrates in a most vivid manner the effect of the absence of the thyroid gland. This salamander never develops beyond the larval stage, retaining permanently its external gills and other larval organs. An examination of the endocrine system of this animal was made by Emerson (13); it revealed the complete absence of the thyroid gland. It is worth while to mention briefly another interesting condition observed in this animal, namely the almost complete lack of pigment, a condition somewhat similar to that observed by Smith and by Allen in hypophysectomized tadpoles, and the highly atrophied state of the eyes. *Typhlomolge* is a white, blind salamander. These latter peculiarities have been attributed frequently to the absence of light in the caves, a theory which at first seems very plausible. It would not be surprising, however, if some day these characters should be found to be the result of endocrine disturbances. Similar to *Typhlomolge* in all the characteristics mentioned above is a European salamander, *Proteus anguineus*, which inhabits the Austrian lime-

stone caves; nothing, however, is known about the endocrine glands of this animal.

If the thyroid substance is capable of causing the development of the characters of a terrestrial amphibian, the administration of thyroid substance should cause metamorphosis of *Proteus anguineus*. Jensen (14) subjected *Proteus* to the action of thyroid substance, but did not get any demonstrable results. Many causes may have been responsible for this failure, in particular the fact that the animals were too old when they were subjected to the thyroid feeding.

It has been known for some time that the effect of equal doses of thyroid substance on the amphibian metamorphosis is the greater, the more iodine there is contained in the thyroid gland (15). Recently, Swingle (16) has demonstrated that the feeding of common inorganic iodine to tadpoles or the keeping of the tadpoles in iodine solutions accelerates metamorphosis in the same way as does the thyroid. This effect of iodine is strictly quantitative; if there is no iodine contained in the food of the tadpoles, metamorphosis is inhibited, while with an increasing amount of iodine metamorphosis is increasingly accelerated. Moreover, the effect on the relation between growth and development is the same in iodine solutions and in thyroid feeding. Weak solutions of iodine increase not only the rate of development, but also the rate of growth, while high concentrations prevent growth. There can be no doubt that at least in the metamorphosis of tadpoles, iodine is an indispensable constituent of the thyroid hormone.

Swingle (16) found that potassium iodide and iodoform had an effect on metamorphosis similar to that of iodine, while bromine had no effect on metamorphosis and growth. Thus the effect of iodine appears to be very specific when comparison is made with so nearly related a substance as bromine.

The feeding of iodine to mammalians does not produce the same effects as the administration of thyroid sub-

stance. This fact has formed the basis for the opinion (7) that the characteristic action of the thyroid hormone is not directly caused by the presence of iodine in the thyroid hormone. It seems, however, more probable that the feeding of iodine has no effect on mammalians, because the mammalian organism, for some reasons, can not utilize an excess of iodine. It is well known that the mammalian thyroid gland is capable of storing large amounts of iodine (17). If, under normal conditions, only a definite amount of hormone could be excreted by the thyroid gland, the feeding of excess amounts of iodine would have no effect in the healthy individual, since every excess of iodine would be retained and stored by the thyroid tissue. If in the mammalian organism the thyroid gland should be the only organ capable of elaborating the thyroid hormone, the feeding of iodine could have no effect in the absence of the thyroid, or in persons whose thyroid function is insufficient. Conditions are different with tadpoles. Swingle (16) has shown that even in thyroidectomized tadpoles, iodine solutions are capable of causing metamorphosis. Apparently the thyroid gland is not the only organ of the tadpole which can produce the thyroid hormone.

It should be pointed out, however, that a fundamental difference exists between frogs and toads on the one hand, and salamanders on the other, as regards their reaction to iodine. Salamanders behave much like mammalians. Although I was able to confirm the accelerating action of iodine at least in the development of the limbs of the tadpoles, I have not been able to cause precocious metamorphosis by placing salamander larvæ in iodine solutions. Table II will illustrate this statement.

Two larvæ of the species *A. maculatum* were kept first in a solution of 5 drops $\frac{1}{20}$ m. iodine per 1,000 c.c. water and then, up to metamorphosis, in a 3-drops-iodine solution. No acceleration of metamorphosis took place; the larvæ metamorphosed at an age of 122 days, while the controls were only 101 days old when they metamor-

phosed. It is interesting to note that while tadpoles of *Rana sylvatica* are killed by a 5-drops-iodine solution and upon a 3-drops-iodine solution respond promptly with development of the hind limbs, the larvæ of *A. maculatum* showed no other effect from a 5-drops solution than a slightly decreased food intake. The latter circumstance may account for the longer duration of the larval period of the experimental larvæ. Since it was believed that in this experiment the solution was too weak, 2 larvæ of the same species, after a short sojourn in a 3-drops solution, were placed in an 8-drops-iodine solution; but as Table II shows, in this experiment also metamorphosis was not accelerated by the iodine solution. Several larvæ were fed directly crystals of iodine to make sure that the ineffectiveness of the iodine solution in salamanders was not due to a possible impermeability of the larval skin for iodine. In one case two crystal-fed larvæ metamorphosed at 124 days, while the controls metamorphosed at the age of 101 days. In another experiment, in which 3 larvæ were employed, one metamorphosed at the age of 89 days, while the controls metamorphosed at 80 days. Of the two other larvæ, one did not show any signs of metamorphosis when it was killed for histological examination; the other one died from an overdose of iodine, but did not show any sign of metamorphosis.

TABLE II

IODINE HAS NO EFFECT ON THE METAMORPHOSIS OF *A. maculatum*

Quantity of Iodine.	Age at Metamorphosis.		
	Normal.	Iodine Solution.	Iodine Sol. + Crystals.
5 to 3 drops 1/20 m. iodine in 1,000 c.c. water	101 days	122 days	124 days
5 to 8 drops 1/20 m. iodine in 1,000 c.c. water	80 "	79 "	89 "

Three old larvæ, all of the axolotl type, and one neotenous, of the western race of *Ambystoma tigrinum*, which were collected in the Rocky Mountain lakes last

summer, were subjected to an iodine treatment. They were placed in water containing 5 drops of a ½₀ m. solution of iodine per 1,000 c.c. of water and, as they showed no reaction of any kind, this concentration was increased gradually to 8 drops and in one larva to even 13 drops of iodine (3 drops of a ½₀ m. solution of iodine per 1,000 c.c. of water is enough to cause growth of the hind limbs in larvæ of *Rana sylvatica*), which is more than 0.2 c.c. of a ½₀ m. solution of iodine per 1,000 c.c. of water. Although these larvæ have now been in the iodine solution for 2 months, none of them has developed any tendency towards metamorphosis, while 3 other control larvæ, among them a neotenous specimen, metamorphosed 13 days after being placed in an emulsion of 0.1 gm. of Bayer's iodothyrine per 1,000 c.c. of water. Evidently the assumption suggested by Swingle (16, III), that lack of iodine prevailing in the lakes is causing the inhibition of metamorphosis of the axolotl and other urodelans, is unwarranted. I will show presently that in the inhibition of metamorphosis and in neoteny of axolotls and probably certain European urodelans we are confronted with an entirely new phase of internal secretion, namely with the differential action of temperature upon the development of various components of the endocrine system.

In a former article (20) I suggested that the inhibition of metamorphosis in thymus-fed amphibian larvæ may be caused by lack of iodine in the thymus. Swingle (16, III) has accepted and unfortunately repeated, without further testing, this suggestion. But recent experiments show that this view must be abandoned, since addition of iodine to a pure thymus diet does not enable the salamander larvæ either to grow or to metamorphose. Similarly the retardation of growth and metamorphosis of salamander larvæ kept on a pure diet of posterior lobe of hypophysis remains unaffected if iodine is added to the water.

The iodine requirement of salamanders must be extremely slight, since anterior lobe of hypophysis, a nearly

iodine-free diet, does not in any way retard growth or metamorphosis.

There are several species of salamanders (*Autodax lugubris, Autodax iecanus*) whose young do not emerge from the eggs before metamorphosis is completed. Although the larvæ of these species have no opportunity to obtain iodine from outside, these cases do not prove, of course, anything against the importance of iodine in the amphibian metamorphosis; very likely the eggs of *Autodax* contain enough iodine to permit metamorphosis of the larvæ within the egg.

Still another difference between anurans and salamanders has made itself apparent in this work. While in tadpoles, of at least certain anuran species, the development of the legs is, in some as yet unknown way, distinctly under the control of the thyroid, the leg-development in salamanders is independent of the thyroid gland. Both hind and fore limbs develop in a normal way after thyroidectomy in salamander larvæ, as shown by E. R. and M. M. Hoskins (10). Moreover, the development of the legs is not accelerated if the larvæ are kept in solutions of iodothyrine (18); this is the case even if the administration of iodothyrine is commenced soon after the eggs have been deposited. Consequently, it is very common to find that the larvæ metamorphose in the iodothyrine solution before the legs are completely developed. It is evident that in tadpoles part of the larval development is controlled by the thyroid function, since neither the hind limbs, from a certain stage on, nor the fore limbs can develop in the absence of the thyroid (9, 10, 19). Apparently the anuran thyroid gland begins to secrete already in the larval period. In salamanders the larval development seems to be highly independent of the thyroid function and it is quite probable that the salamander thyroid does not begin to function much before the first moult. This can be demonstrated in the following way (12). If eyes of old larvæ which, however, are still far enough from metamorphosis, are grafted on to young

larvæ, their metamorphosis is inhibited until the host metamorphoses. If the eye graft, however, is taken from larvæ which are near metamorphosis, such an inhibition is no longer possible. Apparently shortly before metamorphosis actually occurs, the thyroid begins to excrete, and after the circulating hormone has reached the eye metamorphosis of the eye takes place, even if the organ is transferred to an animal in which the thyroid hormone has not yet been secreted.

It is quite possible, that the late beginning of the thyroid function in salamander larvæ is one of the causes why the administration of an excess of iodine is ineffective in the metamorphosis of these amphibians. Probably the thyroid merely stores up the excess of iodine, but does not release the hormone till shortly before the first moult.

Allen (19) has recently examined the condition of the thyroid of Colorado axolotls and has found that they possess a thyroid corresponding in size, structure and colloid content to the thyroid of adult specimens of *A. tigrinum*. The thyroid of the larvæ of other salamander species likewise seems to be mature much before metamorphosis actually takes place. Allen concluded from his observations that the thyroid of salamanders begins to function at an early stage of the larvæ. The independence of the larval development of the salamander larvæ as demonstrated by the facts mentioned above shows, however, that the presence of a mature thyroid before metamorphosis must be interpreted in a different way. The most conspicuous character in the salamander metamorphosis is the fact that, although it certainly is dependent on the thyroid hormone, it does not necessarily take place in larvæ whose thyroid is mature. This can only mean that two factors are required in order to bring about the metamorphosis of salamander larvæ, namely a mature thyroid gland and a factor which releases the thyroid hormone from the follicles of the gland.

This conception, which is now supported by several

facts, is also capable of explaining the problem of neoteny of the so-called axolotl. In the course of experiments carried on during several years in the laboratory, and by inspection of the conditions prevailing in the Rocky Mountain lakes, the natural habitat of the American axolotl, I have become convinced that the neoteny of this species is due to the effect of low temperatures. We have in the amphibians an experimental material in which the relation between the development of the body and certain endocrine glands can be changed by the influence of temperature, owing to the differences of the temperature coefficients of the processes governing the development of different glands.

Although my experiments are not yet finished, they seem to permit the following conclusions in connection with my field observations:

1. The thyroid gland of salamanders undergoes a developmental change consisting of two periods, one of early development, lasting at least 63 weeks, in the course of which the thyroid becomes more and more sensitive to the action of a releasing factor (called excretor substance in my earlier work) and one of aging in the course of which the thyroid loses gradually its sensibility to the releasing factor.

2. In order to release the hormone of the thyroid gland, a particular releasing factor is required (the nature of which is entirely unknown); the quantity of this factor necessary to release the thyroid hormone depends on the sensitivity of the thyroid gland. Metamorphosis can take place only if the thyroid is sensitive and is acted upon by the proper quantity of the releasing factor.

3. The temperature coefficient for the elaboration of the releasing factor is higher than the temperature coefficients for growth and the thyroid change.

The following facts seem to warrant these assumptions:

1. Salamander larvæ, kept at an identical temperature, are nearly all of the same size when they metamorphose. Larvæ kept at low temperatures grow considerably larger

than those kept at high temperature, before they can metamorphose. This is shown in Table III (2). The temperature coefficient for the releasing factor is higher than that for growth.

TABLE III

TEMPERATURE AND SIZE OF THE METAMORPHOSING LARVÆ

Species	Series	Size in Mm.		Series
		25° C.	15 C.°	
Opacum	A 1916	57	67	C 1916
	XIV 1918	61	71	XVIII 1918
Tigrinum	S 1917	102	119	U 1917
	XLVI 1919	103	122	XLVIII 1919
Maculatum	LXXV 1920	52	59	LXXVII 1920

2. In very low temperatures (6° C. to 10° C.) growth is greatly slowed down and consequently the elaboration of the releasing factor must be still more retarded; yet larvæ kept at 6° C. grow less and less large before metamorphosis, when they are transferred, at increasing ages, to 15° C., as shown by an experiment lasting 63 weeks thus far. Apparently the thyroid has gone on to mature at a relatively high rate and at 63 weeks is highly sensitive and responds to smaller quantities of the releasing factor. The temperature coefficient for the thyroid change is considerably lower than those for growth and for the elaboration of the releasing factor.

3. If the thyroid can continue to develop in the absence of growth, it probably can also commence to age. Should this assumption be correct, the larvæ kept at 6° C. should finally become unable to metamorphose, if the time during which they are kept in 6° C. is sufficiently long. At present this assumption would explain why many specimens of the Colorado axolotl yield only slowly, if at all, to the influence of high temperature, and the Mexican axolotl frequently loses completely its ability to metamorphose.

4. The Colorado axolotls reach frequently a size considerably in excess of the normal maximum size of the

species as calculated from the largest known terrestrial specimens of the eastern race of this species; the Colorado axolotls are giants. Since sexually mature specimens of the eastern race of *A. tigrinum* become giants if they are fed anterior lobe of hypophysis, the gigantism of the sexually mature axolotl could be explained if any indications of hyperpituitarism of these animals could be discovered. On the assumption that in spite of the presence of a large thyroid the function of this organ is suppressed by the absence of the releasing factor, the overfunction of the axolotl hypophysis would be very plausible, since, as will be pointed out later on, the absence of the thyroid function causes hypertrophy of the hypophysis in amphibian larvæ.

5. The maturing of the sex organs of the axolotl is not incompatible with the assumption of an athyroidism, since, as will be discussed presently, there can be no longer any doubt that the development of the sex organs of amphibians is entirely independent of the thyroid hormone.

6. The assumption that the temperature effect can actually produce the complex phenomenon of neoteny is supported by the fact that the species *A. tigrinum* becomes neotenous only in the high and cold regions of the Rocky Mountains and the Mexican high plateau, while in the eastern part of the United States all individuals of this species metamorphose in a normal manner. I have examined the conditions prevailing in the Rocky Mountains; to summarize briefly my observations, the axolotl is regularly found only in those lakes which are permanently exposed to low temperatures, while in the shallow lakes of lower altitudes axolotls are found only during some years and are absent during other years; apparently a succession of several years favorable in temperature conditions is required to produce the axolotl state.

7. *A. tigrinum* is the only species of North American salamanders which becomes neotenous. This is probably

not due to differences existing between the endocrine system of the numerous species inhabiting the United States, but is explained by the fact that *A. tigrinum*, among the closely related species which I had an opportunity to test, is the only species that can stand temperatures low enough to bring about the necessary difference between the rate of the thyroid development and that of the elaboration of the releasing factor.

8. The fact that many individuals among the offspring of female specimens of the Mexican axolotl do not metamorphose even if they are brought, immediately after hatching, into conditions permitting normal metamorphosis of other salamander species, is not necessarily related to the factors discussed above, but may be due to the development of congenital thyroid disturbance in the young born by an athyroidous female.

It is, of course, well known that many structural changes, only a few of which have been studied, are required to make, out of the aquatic larvæ, the terrestrial amphibian. This is true for the anurans as well as for the urodelans. Since we know that the complex phenomenon of metamorphosis is initiated by the thyroid effect, the question arises now which of the component changes are directly caused by the action of the thyroid hormone. The fact that certain developmental processes frequently take place upon thyroid administration and therefore are a very convenient indicator in studying quantitatively the effect of thyroid substance, of iodine or of any other metamorphosis-causing agent, does not mean, in itself, that these developmental processes are caused directly by the action of the thyroid; it is possible and indeed supported by many facts, that certain of these changes will follow automatically, after the initial changes have been effected by the thyroid action. Thus, while under normal conditions, the pigmentary pattern, the legs, the tongue, the palatal teeth and the sex organs mature in salamanders during metamorphosis, they can be shown to be highly independent of

the thyroid action at least in salamanders and may, under certain conditions, occur in the absence of this action or not occur in the presence of it. I have pointed out in former articles (18) that among the many changes occurring during the salamander metamorphosis there are two which seem to be particularly closely related to the thyroid action, namely the first shedding of the skin and the reduction of the gills to mere stubs. While the succession of all the other changes enumerated above seems extremely variable, the order in which the first moult and the reduction of the gills follow each other could not be changed as yet by any of the procedures employed, inasmuch as the shedding of the skin always is followed by the atrophy of the gills. Moreover, these two phenomena have never failed to occur in the metamorphosis of the many hundreds of metamorphosing larvæ observed in the laboratory, and even in such larvæ as were forced at a very early date into precocious metamorphosis by the administration of iodothyrine and in which other changes did not occur. And furthermore, neither the first moult nor the reduction of the gills could ever be observed in larvæ, whose metamorphosis was inhibited by dietary or other means. Thus I have come to look upon the first moult and the atrophy of the gills as two of the primary components of the salamander metamorphosis. I have not enough personal experience with the larvæ of anurans, but feel encouraged through the experiences reported by other investigators to believe that in anurans these phenomena play a similarly important rôle. Certainly the first shedding of the skin seems to accompany true metamorphosis in salamanders and tadpoles as well (38), and substances other than thyroid hormone or iodine, such as the anterior lobe substance, although they may cause the limbs to grow, do not bring about atrophy of the gills in thyroidectomized tadpoles (27).

It is different with the limbs, the pigmentary pattern, the tongue, the palatal teeth and the sex organs; these five groups of organs, at least in salamanders, have

proved to be little influenced by the thyroid action. That the development of the limbs of salamanders is not dependent on the thyroid gland has been pointed out above; here I may add that Typhlomolge is a further illustration of this fact, as in this salamander the legs develop in a normal manner in spite of the complete absence of the thyroid gland. The relation of limb development and thyroid action in tadpoles is by no means definitely settled as yet. In tadpoles the development of the limbs seems to be highly dependent on the action of the thyroid gland; but attention has been called to this surprising difference between two groups of organisms so closely related otherwise and the suggestion has been made in a previous article (18), that this difference as far as the fore legs are concerned may be due merely to the fact that in tadpoles the limbs grow beneath the skin and consequently can not break through unless the changes are initiated which finally lead to the shedding of the skin and that these changes and not the thyroid action are the primary factor in the development of the anuran fore limbs. Whether or not this assumption is correct can not be decided at present, but certainly deserves renewed attention in view of recent discoveries which demonstrate that the development of the limbs of tadpoles, at least in certain species, is not as dependent on the thyroid secretion as some investigators were inclined to think. Allen (34), who has made prolonged observations in thyroidectomized tadpoles, has recently found that not only the hind limbs, but even the fore limbs in the thyroidectomized larvæ of *Bufo* ultimately attain a size and differentiation not only equal but superior to those attained in normal metamorphosing larvæ. The only difference, however, is that in the absence of the thyroid gland the fore limbs can not break through the skin.

As to the skin pigmentation, it is well known that larvæ in which metamorphosis has been inhibited for some reasons may develop a nearly adult pigment pattern. In larvæ of *A. opacum* which were fed thymus gland, and

consequently did not metamorphose, the coloration of the skin advanced to a stage very similar to that of an adult animal. On the other hand if young larvæ of *A. opacum* are made to metamorphose precociously by means of the application of iodothyrine, metamorphosis takes place, while the color pattern remains in an early larval stage. Through the observations of Cope (35) it has become known that otherwise completely metamorphosed individuals of the species *A. tigrinum* may exhibit either a larval condition of the tongue or larval characters of the palatal teeth or larval characters in both the tongue and the palatal teeth.

In nature it is not uncommon that the sex glands of salamanders develop to complete maturity while the rest of the organism remains in a larval stage (18). This phenomenon, known by the name of neoteny, illustrates that the sex organs can develop in the absence of the thyroid function. The same fact has been shown in the larvæ of anurans by B. M. Allen and his coworkers. In thyroid-fed frog larvæ, which have undergone precocious metamorphosis, the sex organs do not seem to be further developed than those of normal larvæ of the same age (3). Moreover, if the thyroid is removed from the larvæ and metamorphosis inhibited, the sex organs develop at the same rate as in normal larvæ (21). Hoskins (22) and Allen (21) showed that the testicle of thyroidectomized tadpoles may develop ripe spermatozoa. These facts, however, can not be interpreted to mean that the germ plasm is independent of the somatic plasm, in the Weismannian sense. The characteristic feature of the amphibian development is not the independence of the germ plasm from the somatic plasm, but the independence of various groups of organs from one another, due to the fact that the development of each of these groups is controlled by substances different from those controlling the other groups, and that each of these substances separately may be supplied to or withheld from the organism either by the experimenter or by conditions

not fully known as yet (18). One of these conditions is the temperature as has been pointed out above.

I will discuss briefly now the rôle of the hypophysis in the growth and development of amphibians. The most noteworthy fact seems to be the existence of a remarkable resemblance between the functions of the amphibian thyroid and hypophysis glands during the larval period. If the hypophysis gland is extirpated in early embryonic stages, the tadpoles stop to develop at a stage at which, in normal tadpoles, metamorphosis begins. Growth, too, is inhibited in the hypophysectomized tadpoles (23, 24). In a series of extremely interesting experiments Allen (25) showed that both growth and development can be restored to the hypophysectomized tadpoles, if the anterior lobe of the hypophysis of an adult frog is grafted to such larvæ. No other part of the hypophysis when grafted to the hypophysectomized tadpoles can restore growth and development, and it is certain, therefore, that it is the anterior lobe of the hypophysis which controls the growth and development of the larvæ. In tadpoles the feeding experiments as made by P. E. Smith (26) seem to corroborate the extirpation experiments. Feeding of anterior lobe to hypophysectomized tadpoles increases the rate of growth to such an extent that growth becomes as vigorous as in normal larvæ. Moreover, at the time when the normal tadpoles metamorphose and growth ceases for a time, the anterior lobe-fed hypophysectomized tadpoles continue to grow and finally attain a size in excess of that of normal larvæ. Ultimately, however, the growth of these larvæ stops and before the size is reached characteristic of the normal adult animal. The effect of feeding anterior lobe to normal larvæ is a matter still under discussion at present. Smith (26) found that normal tadpoles when fed anterior lobe grew apparently at a slightly higher rate and also metamorphosed at a slightly earlier date than normally fed tadpoles. Recently, however, Smith (36), on account of the considerable variation in the rate of growth and develop-

ment of normal larvæ, seems to be inclined to consider these differences as being of no significance. Certainly it is of no small importance that normal and hypophysectomized larvæ react so differently to a diet of anterior lobe substance; apparently part of the active principle of the anterior lobe introduced, by the diet, into the organism is made ineffective in the presence of a normal hypophysis. Not yet completed experiments on salamander larvæ seem to suggest that the larval growth of salamanders at least can not be affected by feeding anterior lobe of hypophysis; this may be due either to a destruction of the active principle in the digestive tract or to some peculiarity in the metabolism of the salamander larvæ, and is of particular interest with regard to the fact that the adult salamanders react very markedly to an anterior lobe diet, as will be discussed presently.

One of the most pertinent and yet most difficult problems of endocrinology is presented by the existence of interrelations and interactions between the various endocrine glands. There can be no doubt that in tadpoles such an interrelation exists between the hypophysis and thyroid glands. Thus Rogers (31) and later on Hoskins and Hoskins (22) found that upon thyroidectomy performed in early embryonic stages of anurans the anterior lobe of the hypophysis shows a tendency towards hypertrophy. On the other hand if the buccal anlage of the hypophysis is removed, the thyroid soon ceases to grow and to differentiate and finally presents a state of hypoplasia, as shown by Allen (30) and by Smith (36). Since the effects of the extirpation of either of these glands on general body growth and on development are quite similar and since the behavior of each of these glands after the extirpation of the other one demonstrates the existence of an interrelation between them, the question might well be asked, if the function of each of these glands can not be replaced by the hormone of the other one of them. Although this question can not be satisfactorily answered thus far, it seems highly probable that these hormones

are strictly specific in as much as neither of them can replace the function of the missing one. To quoting the inhibition of metamorphosis and growth following hypophysectomy as proof in favor of this view one could object that in this particular case the thyroid can not effect metamorphosis and growth merely on account of its atrophic condition. Smith (36), however, found, that in certain cases of partial hypophysectomy the thyroid remains completely unaffected and yet no metamorphosis takes place; only if the remaining fragment of the epithelial hypophysis grows large enough to come in contact with the neural hypophysis, metamorphosis can be effected. For this reason Smith takes the view that the function of the hypophysis is indispensable in metamorphosis and that the secretion necessary for this purpose can only be elaborated, if epithelial and neural hypophysis are in contact with each other. That neither the anterior nor the posterior lobe of the hypophysis contains the substance necessary for metamorphosis and that this substance can be produced only in the body itself, requiring for its elaboration the contact between neural and buccal hypophysis, seems much supported by the fact that, although growth may be maintained up to a certain size, by feeding anterior lobe to hypophysectomized tadpoles, metamorphosis can not be effected in such tadpoles by feeding either anterior or posterior lobe. As to the possibility of replacing the function of the anterior lobe substance by introducing into the organism thyroid hormone or iodine, Allen (28) fed iodine to hypophysectomized tadpoles and obtained some, but not all of the changes induced by iodine in normal and thyroidectomized larvæ and seemed to be tardily inclined to the view that the lack of the hypophsis could be compensated for by feeding iodine. Smith, however, in his last publication (36), claims that neither thyroxin nor thyroid gland itself causes metamorphosis, when fed to pituitaryless tadpoles. Quite similar are the results of feeding hypophysis to thyroidectomized larvæ. Hoskins and Hoskins (27) were able to cause growth of limbs and emaciation

by feeding anterior lobe substance to thyroidectomized tadpoles, but could not obtain complete metamorphosis; especially the atrophy of the tail and of the gills could not be enforced. Similarly Allen (37) points out that feeding anterior lobe of cattle does not result in metamorphosis of thyroidectomized tadpoles.

If taken together, all these results seem to indicate that although certain resemblances exist between the hormones of the thyroid and the hypophysis glands, they are nevertheless specific and can not replace each other as regards at least certain functions.

As pointed out above, the metamorphosed salamanders react on anterior lobe feeding quite differently from the larvæ. Such differences in the reaction upon the same principle in different stages have been observed quite frequently and are apt to throw an important light on the nature of the chemical reactions involved in growth and development of different stages. The salamander larvæ show no appreciable effect from an anterior lobe diet, whether the anterior lobe be fed alone or in small quantities added to normal food. If metamorphosed salamanders of the species *A. opacum* or *A. tigrinum* are fed anterior lobe, the rate of growth becomes almost immediately accelerated and growth continues after the animals have reached the specific maximum size of the species; they become giants. The latter result must be attributed to the action of a specific growth promoting hormone contained in the anterior lobe (32).

The thymus gland apparently has no effect on growth and development, although it has been believed that it contains specific growth-promoting and development-retarding substances. It is true that in larvæ which are fed on thymus only, growth as well as metamorphosis are inhibited. The inhibition of metamorphosis, however, is due to the fact that in the absence of growth the releasing factor of the thyroid can not form, as has been mentioned above. Moreover, the inhibition of growth is not caused by specific hormones of the thymus, but is merely a deficiency phenomenon. The more normal food

there is added to the thymus, the less marked does the inhibition of growth become; small amounts of thymus added to a normal diet have no effect (33). It is unknown at present which of the food substances necessary for growth are missing, although it is certain that the deficiency of the thymus is not caused by a deficiency in iodine, calcium, sodium or potassium. Many other glands, such as the spleen, prescapular lymph-gland, parathyroids, and posterior lobe of the hypophysis are more or less deficient in the growth of salamander larvæ.

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CONTRIBUTION TO THE KNOWLEDGE OF THE NUDIBRANCHIATE MOLLUSK, MELIBE LEONINA (GOULD)*

PROFESSOR H. P. VON W. KJERSCHOW-AGERSBORG[1]
UNIVERSITY OF WYOMING

INTRODUCTION

THE writer's attention was drawn to this rather unusual type of molluskan life (*Melibe leonina*) in observing the living animals at the Puget Sound Biological Station, located at Friday Harbor, Washington, during the summer of 1914, and inasmuch as little was known concerning the species, an effort was made to assemble such data as might be of interest relative to its habits and development. The results of this study are presented in the following pages.

It was originally intended to publish these in connection with work on the morphology of the species, now under preparation, but owing to the extent of the morphological data and the crowded condition of the morphological journals it seems best to have this other matter appear separately.

TAXONOMY

The genus *Melibe* (Rang), together with *Tethys* (Linné), constitutes the family *Tethymelibidæ*, which forms one of the numerous groups of family rank included in the sub-order *Nudibranchiata* of the opisthobranchiate *Mollusca*. The type of the genus *Melibe* was discovered at the Cape of Good Hope and described by Rang in 1829. Since that time eleven species have been added by various

* Received by the Editor on May 5, 1920.

[1] In my previous writings, *Biol. Bull.*, Vol. 35, No. 4, 1918; *School and Society*, Vol. 9, No. 232, 1919; Pub. Puget Sound Biol Sta., 2, No. 49, 1919; AMERICAN NATURALIST, Vol. 54, 1920, my name is written Kjerskog- . . . which is the modern form of the original (Kjerschow) substituted henceforth.

authors. Gould, 1852, described *Melibe leonina*, the species upon which this paper is based, from Puget Sound, founding for it the genus *Chiorœra* now merged in *Melibe*. Bergh, in a series of papers between 1863 and 1907 revolutionized the classification of the *Nudibranchiata*. He divided the nudibranchs into two sections: the *Kladohepatica* and *Holohepatica porostomata*. Although his work was primarily systematic, it was based on morphological studies. He added six species to the genus *Melibe*, including *M. pellucida*, from the coast of Washington near the mouth of the Columbia River. His description indicates such a close similarity to *M. leonina* that it may be questioned whether this species is entitled to specific rank; material from the type locality may be necessary to settle this point. The more recent work on taxonomy by Sir Charles Eliot, 1910, modifies Bergh's work to some extent.

No detailed study has been made of *M. leonina* previous to the present work and our knowledge of the species rested largely with the brief description and figure presented by Gould.

Distribution

Melibe, as far as known, is restricted to the Pacific and Indian Oceans, and to the South Seas. On the eastern

FIG. 1. *Melibe* (*Chioraera*) *leonina* Gould. From a drawing by Mr. Bert Elliot, private artist to Professor Trevor Kincaid, University of Washington. Slightly changed. × 1/3.

FIG. 2. Photograph, by author, of preserved specimen of *M. leonina* showing profuse branching of hepatic system in the body-wall; the hood is contracted into a knob as when the animal swallows. Slightly reduced.

side of the Pacific, two species have been described, *M. leonina* Gould, 1852, and *M. pellucida* Bergh, 1904. On the western side of the Pacific, various species have been found by Bergh from the Japanese Island to the Straits Settlements. One species has been described by Angas, 1864, from Australia; one from the Pacific by De Filippi, 1868; one from the Sandwich Islands by Pease, 1860; and several species from the Indian Ocean by Rang, 1829, Alder and Hancock, 1864, and Eliot, 1902. So far no species of this genus seems to have been found on the coasts of the Atlantic Ocean. In these parts of the world, and in the Mediterranean and Caribbean Seas, it seems, according to works of Bergh, 1877, 1890*a*, Locard, 1885, Viguier, 1898, and Eliot, 1910, to be replaced by *Tethys* Linné. Eliot 1910 (pp. 12–13), fails to record *Melibe* among the American Pacific fauna. Neither *Tethys* nor *Melibe* seems to occur in the northern waters. All the Melibidæ recorded by Agassiz, 1852, G. O. Sars, 1878, Johnston, 1838 (*Melibea*), are, in fact, not *Melibe*, but

Doto. Von Marten's (1879) *Melibea* does not seem to come under either type, although from this author's description certain characteristics are in common with that of *Melibe.* For some time there was considerable confusion in regard to these genera. Bergh, 1863, and again in 1871, makes this clear when he writes:

> Das Geschlecht Doto wurde von Oken (1815) auf der Gemlin'schen *Doris coronata* (Bomme) aufgestellt. Jahre nachher (1829) bildete Rang (Man., p. 129, pl. 3, f. 3) eine neue Geschlechtsform Melibe ab, dessen Typus eine Nacktschnecke war, die er im Meere des Vorgebirges der guten Hoffnung (Cap) am schwimmenden Meeresgrase fand. Das Rang'sche Geschlecht, das von späteren Verfassern gewöhnlich Melibaea genannt ist, ist am meisten mit dem Oken'schen Doto identificirt worden, in der Art, dass alle bisher bekannten Melibaeen—eben mit Ausnahme der "Melibe" rosea von Rang—der Doto-Gruppe gehören. Die Melibaea, autt. sind in der That mit den Doto'en identisch und mussen diesen Namen bekommen. Die Meliben Rangs dagegen werden, wie früher von mir hervorgehoben (Schiödte Naturh. Tidskr., 3 R, I, 3, 1863, p. 480), einen ganz verschiedenen Geschlechstypus bilden, den ich als mit den Chioraeren von Gould verwändt betrachtete, der aber einer späteren Mittheilung von Alder und Hancock (1864) zufolge den Scyllaeen näher (?) käme.

The genus *Tethys* Linné, 1758, the nearest relative of *Melibe* Rang, 1829, was also confused with other forms, *e.g., Aplysia.* Pilsbry, 1895, has, however, cleared up this point.[1] He shows that the various specific names attached to *Tethys,* such as *fimbria, fimbriata,* and *leporina,* are to be considered synonyms. Bergh, 1880*a,* and Krause, 1885, do not record *Melibe* in their nudibranchiate collections from the north Pacific. Likewise, investigators in the north Atlantic, on both sides of the ocean, including a number of explorers and independent workers such as Alder and Hancock, 1842 and 1845; Meyer and Möbius, 1865; M. Sars, 1870; Aurivillius, 1885; Garstang, 1890; Krause, 1895; Ohdner, 1907; Walton, 1907; Théel, 1908; and Johnson, 1915, do not record *Tethys* or *Melibe* in their collections. These species are, therefore, restricted to the warmer seas.

When the Puget Sound Biological Station[1] was estab-

[2] This station was first known as the Puget Sound Marine Station; its founder, Professor Trevor Kincaid, remained its director until 1914.

lished in the San Juan Archipelago in 1904, it was found that *Melibe leonina* was not uncommon in the vicinity, although like many pelagic organisms its abundance was subject to great fluctuations. In the summer of 1912 it was particularly abundant, great numbers appearing among the fronds of *Nereocystis* drifting past the floating dock in front of the station. At this time Prof. H. L. Osterud, of the University of Washington, gathered and preserved a considerable number of specimens. The largest specimen obtained was six cm. in length, the time of collecting being the latter part of July. During the season of 1913 very few were seen. In 1914, the writer found several specimens of large size, 8 to 13 cm. in length. These were taken among the floating eel-grass, *Zostera marina*. In the summer of 1915 only two specimens were found. It appears that the genus *Tethys* is of spasmodic recurrence in the Mediterranean (Viguier, 1898). The appearance of *Melibe* does not seem to be determined by any particular season, as Prof. Osterud found specimens spawning when visiting the Biological Station early in March of 1916. The period of existence of this nudibranch must be more than one year, or one season, judged from the sexual condition in this species which has shown maturity in individuals ranging in size from two to fourteen centimeters. Alder and Hancock (1845), p. 24, think the period of existence for a large number of species "not much exceeding one year."

ECOLOGY

There is a striking similarity between the *Kladohepaticæ*, not only between the members of any given family, but also between members of different families within the section. The former is well illustrated by *Tethys* and *Melibe*, members of the family Tethymelibidæ. This similarity is not only morphological, but equally true as to manner of living and general behavior, for instance, the method of swimming. *M. leonina* may crawl on the leaves of the eel-grass; or it may float with

the back up, the hood having air under it, or the papillæ serving as floats. In the latter case *Melibe* alternately bends laterally the anterior end to angles of 45 degrees. During these alternating bendings of the body the movements are swiftest when the body is relaxed from the 45-degree bend. By this method it conveys itself slowly through the water. However, on the surface tension of the water or on the eel-grass, the movements are caused by the ciliary action of the ventral surface of the foot, because progressive movements occur without visible bodily contortion. *Tethys leporina* Linn. moves through the water by similar means (Bergh, 1877 and 1883). In its case, however, the large veil, as well as the lateral bendings of the body, plays an important part. Gould, 1852, in his original description of *Melibe leonina*, says:

> This animal swims by lateral flexions of the body, the foot being then folded; and when crawling it is able to flex its enormous head laterally with considerable force (p. 310).

Scyllæa pelagica Collingwood, 1879, swims very much like *M. leonina* and *T. fimbria;* and Garstang, 1890, mentions that *Lomanotus* Vérany, swims "vigorously through the water in the dish . . . by lashing the body from side to side." But neither Gould, Bergh, Collingwood, nor Garstang mentions ciliary action as a factor in locomotion.

Collingwood, 1879, says in part:

> Considerable numbers of this pelagic species were found upon *Sargassum bacciferum*, floating in Lat. 25 N., Long. 37 W., most species of weed having one or more specimens. The animals were in constant movements of contracting and writhing. In the water they swam freely, moving the head and tail from side to side alternatingly, so as nearly to touch one another; and when thus swimming were always, owing to the weight of the papillary prolongations and tentacles, back downward, and bore grotesque resemblance to a four-legged animal with ears, such as a Skye terrier.

Another similarity between *Scyllæa* and *M. leonina* is the manner of dropping from the surface to deeper water. In the case of *leonina* this is a sort of death feigning. *S. pelagica* may be found at the surface or in

deeper water. Collingwood mentions it as assuming certain aspects when it falls through the water to a considerable depth, where it is frequently found. *M. leonina* possesses the same habit. In so doing the muscles of *leonina* are absolutely relaxed and the animal appears dead. The means by which the animal gets to the surface are not known, unless it be by its general mode of swimming. One striking difference between these two forms is: *M. leonina* may swim with the back upward, "the weight of the papillary prolongations," important in *S. pelagica,* being apparently of no account.

The eel-grass offers an excellent feeding ground for *Melibe.* Here the water must not only be calm, but may abound in small Crustacea. *Zostera,* which grows in large beds in the bays near the Biological Station at Friday Harbor, offers also a suitable assembling place for *Melibe* where it may pair and lay its eggs. At low-tide the eel-grass floats on the surface of the water and leaves many inclosures of open water. In these open spaces *M. leonina* collect and copulate, as was observed in the summer of 1914. In such spaces, as described, a considerable number of *Melibe* had collected, and some of them were copulating, being united head to head, the foot of one mate facing the surface. The excellent condition of the water offered an ample opportunity to study the mode of swimming and the manner of feeding. The former has partly been described above, and will further be discussed under the topic on observations in the laboratory; the latter corresponds to Eliot's description (1902) of *M. fimbriata* Ald. and Hanc. *M. leonina* is not so definite in its movements during its feeding as is *M. fimbriata,* yet some similar method of feeding is pursued. Both species have a large hood. In the case of *leonina* the hood is extended very widely (Fig. 1), when the animal is searching for food, and is periodically contracted into a knob (Fig. 2), when food is obtained. When the hood is open, *leonina* tosses it sideways, holding it in direct position for the capture of small horizon-

tally swimming crustaceans. Eliot, 1902, says of *M. fimbriata:*

> In spite of its want of jaws, *Melibe fimbriata* is a most voracious animal, and I more than once found in the stomach which I examined limbs of Crustacea more than an inch long. . . . The movements of the animal are rapid and energetic, whether it crawls or swims. It can float on the surface, foot uppermost (p. 70).

Melibe leonina is actively predaceous also; its gizzard has been found completely filled (Agersborg, 1919) with minute Copepoda, Amphipoda, and larger and smaller Isopoda, until the gizzard would bulge out into almost a perfect sphere; ordinarily the gizzard has only a partial enlargement; its normal size is a little larger than that of the proventriculus, and the anterior part of the intestine.

Daugherty and Daugherty, 1912 and 1917 (p. 83), refer to nudibranchs as vegetable feeders; having mentioned *Eolis* and *Pleurophyllida,* they say:

> These soft naked sea-slugs live in shallow water near the shore, crawling about and feeding upon the sea-weeds.

However, only a few of the rarer species are phytivorous; the majority are carnivorous, a fact which is recognized by the authorities on nudibranchiate *Mollusca.* Thus Bergh, in most of his descriptions of nudibranchiate fauna, reports in favor of animal diet: 1880*a* (*Akiodoris lutescens*) p. 56, (*Lamellidoris bilamellata*) p. 64, (*L. luptricina, Acanthodoris pilosa*) p. 101, (*Triopha modesta*) p. 116; the food of these forms consisted of "indeterminable animal matter, mixed with some diatomaceæ, . . . and with some Polytholamia, . . . with larger and smaller pieces of small Crustacea, . . . and a little indeterminable worm, of the length of 2.0 mm., . . . spongiary masses and different Radiolariæ of a diameter of 0.09 mm." In regard to *Tritonia reticulata,* the same author, 1881, says:

> Die Tritoniaden sind Raubthieren und scheinen sich hauptsachlich von Alcyonien und ähnlichen Thierformen zu ernähren.

Again, 1883, referring to *Tethys leporina,* he says:

Tethys ist ein Raubthier und sein Nahrung besteht namentlich aus kleinen Ophiuren, deren Reste oft ganz den Magen erfüllen.

1890*a*:

Der Magen und der Darmen von Nahrung vollgeslofft; dieselbe bestand aus Massen von kleinen Decapoden, mit Bruchstücken von kleineren Gasteropod Schalen und Sandkörnern vermischt (p. 158).

1894 (*Dendronotus robustus*):

In der verdauungshöle unbestimmbare thierische Masse, mit Diatomeen vermischt (p. 144).

And finally, describing the food of *Melibe rosea*, 1907, he says:

The contents of the alimentary cavity (specimen 1.5 cm.–3.8 cm.) were animal matter with remains of small Hydroids (p. 98).

Alder and Hancock, 1845, p. 23, say:

But, though so patient and long-suffering in the endurance of hunger, these little animals are very voracious. The greater number of them are carnivorous; living principally upon zoophytes and sponges. The *Alcyonium digitatum* is a favorite food with the *Tritoniæ;* and the *Actiniæ* and *Lucenariæ* often fall prey to the attacks of the *Eolides.* These latter, indeed, do not scruple occasionally to devour the weaker among their own brethren, as we have recorded elsewhere. Sir J. G. Dalyell states that his *Eolis histrix* (Drummondi) 'fed voraciously on mussel, and on common periwinkle, whereof large portions were swallowed entire'; and he thinks that *Goniodoris nodosa* feeds upon *Ascidia papilla* (*Cynthia rustica*), to which he attributes the reddish colour observed in the viscera. This colour, however, is caused by the liver and ovary. We have taken from the stomach of *Eolis papillosa* minute specimens of the common mussel, and a small *Terebra* from that of Tethys. The more common food of the tribe, however, is the flexible zoophytes. Until lately the Dorides have been considered vegetable feeders, but this would appear not to be the case. *Doris tuberculata* feeds upon common encrusting sponges (*Halichondria panicea*), and sponges and zoophytes seem to constitute the food of most of the others. A few of the gregarious Nudibranchs, such as *Polycera quadrilineata, Hermæa dendritica,* and *Alderia modesta,* which congregate on marine algae, appear to be phytivorous; but *Eolis despecta,* and *E. exigua* though not unfrequently gregarious on the fronds of *Laminaria digitata,* are only found on those parts of the plants that are covered with the parasitic zoophytes, *Laomedea geniculata* and *L. gelatinosa,* on which they feed and deposit their spawn.

Meyer and Möbius, 1865, are of the opinion that it is

rather difficult to determine whether Nudibranchs are carnivorous or phytivorous, that is, that the food of Nudibranchs is very variable. In part they say of *Elysia viridis* Montagu, p. 10:

> Sie nährt sich wahrscheinlich von Pflanzen.

Page 23:

> *Eolis alba* frisst, wie die anderen Kieler Arten ihrer Gattung, thierische Stoffe.

Page 31 (*Eolis papillosa*):

> Ihre Nahrung sind Thierstoffe; besonders liebt sie Actinien. Kleinere-Exemplare der *Actinia plumosa* greift sie am Fussrande an, und frisst ein halbmondförmiges Loch hinein, das sie immer mehr vergrössert.

This is also the opinion of Hecht, 1895, p. 621:

> Il n'est donc pas possible d'établir à ce point de vue une division bien tranchée. On peut dire seulement, que les familles les plus franchement herbivores sont les Hermaeidae et les Elysiidae, et en général les Ascoglosses qui, comme Ihering et d'autres l'ont remarqué, ont une masse buccale disposée pour exercer une succion. . . .

Page 622:

> Les Eolidiens sont tous franchement carnivores et présentent, parmi les grandes espèces, quelques types d'une voracité extraordinaire. *Eolis coronata* . . . devore des *Elysia viridis;* à l'autopsie j'ai trouvé des radulas dans son tube digestif. Les petites espèces *Eolis despecta, E. exiqua, E. olivacea* peu faites pour de grands déplacements, vivent à demeure, comme je l'ai dit plus haut, sur des colonies d'Hydroides.

Page 223:

> *Calma glaucoïdes,* qui, pendant une période de sa vie tout ou moins, se nourrit, je l'ai dit, d'embryons de Poissons. Le régime des Doridiens est moins uniforme; certains genres sont probablement herbivores. Plusieurs espèces de Doris se nourrissent d'Eponges calcaires dont on retrouve les spicules dans les excreta. . . . Plusieurs espèces de Goniodoris se nourissent de Bryozoaires. Il est probable que *Polycera quadrilineata* mange des Algues. Il faut signaler ici les observations de Prouho, sur la façon particulière dont *Idalia elegans* se nourrit de certaines Ascidies. Quant aux Ascoglosses, j'ai indiqué plus haut que *Hermaea dentritica* dévore les couches superficielles des Codium tomentosum, qu'elle réduit à l'état d'un petit moignon verdâtre. *Elysia viridis* se nourrit aussi de Codium tomentosum, mais sans marquer de préférence pour telle ou telle région; j'ai du reste observé qu'elle s'accommode aussi d'autres Algues.

Jeffreys, 1869, contributes to this subject and says:

Although most of the order are zoophagous, *Limapontia* and others of a simpler kind feed on seaweeds.

And von Ihering, 1876, p. 37, referring to *Tethys* states:

Das der Magen eines so gefrässigen, jeder Bewaffnung des Mundes baaren Raubthieres wie *Tethys* eines solchen Schutzes ganz besonders bedarf, wird sofort verständlich, wenn man den Mageninhalt desselben kennen lernt. Ich fand denselben ausser aus Tangstücken bestehen in zahlreichen Crustaceen, kleinen Echinodermen und mehrmals auch kleinen Fischen, von denen einer 4 cm. lang war. Dasselbe Thier enthielt noch die Otalithen eines andren Fisches, welche diejenigen des ebenbezeichneten um das Doppelte übertrafen.

Melibe fimbriata Ald. and Hanc., is, according to Eliot, 1902,

in spite of its want of jaws, a most voracious animal.

This same author says he more than once found in the stomach he examined limbs of Crustacea more than an inch long. And, in 1910:

Thus the red British Dorids *Rostanza coccinea* and *D. flammea* eat red sponges, such as *Microciona atrasanguinea* (p. 5).

In fact, this author thinks that most Dorids feed on sponges (Eliot, 1910, p. 39). Step, 1901, referring to the crowned sea-nymph, *Doto coronata,* says it feeds upon Hydroids (*Sertularia* and *Plumularia*) and Corrallines (*Antennularia antennina*). The marble slug, *Lomanotus marmoratus,* feeds upon corrallines which it closely resembles in color and ornamentation. Eolis feeds upon anemones, *Sagertia, Lucernaria;* Sea-mats, *Tubularia,* various sponges and *Obelia.* The crimson Hermæa (Ascoglossa), *Hermæa bifida,* feeds on small crimson weeds (*Bryopsis, Codium, Enteromorpha* and *Ulva*). Vayssiere, 1901, p. 84, referring to *Tethys fimbria,* Bohascht, Delle Chiaje (Synon. *T. leporina* Linné, Cuvier), writes:

Dans l'intérieur du premier renflement stomacal (jabot), je trouvais d'ordinaire une grande quantité de filaments fibreux de Zostères; ces mollusques doivent en aspirant avec leur trompe, absorber des débris de

ces végétaux et dissocier leurs fibres par les contractions répétées des parois musculaires de cette poche.

Page 85:

Au milieu de ces débris, ils trouvent de petits crustacés (*Entomostracés, Amphipodes, Isopodes,* jeunes *Décapodes brachyures*) et autres petits Invertébrés, circulant parmi les Zostères, qui doivent former la base de leur nourriture.

Thus it is seen that even the carnivorous *Tethys* may be phytivorous. Vayssiere, 1911, p. 43, says of *Halgerda willeyi* C. Eliot, 1903:

La poche stomacale était remplie de gros débris Crayeux constitués par des fragments de madrépores et de bryozoaires que ce mollusque arrache et broie avec sa forte radula.

Finally, MacFarland, 1912, p. 530, says in regard to the Dironidæ (*Diron allolineata*):

Diatom shells and minute spicules, these made up a very small portion of the total contents.

Nudibranchs may be said to be omnivorous; as seen above, a species which is void of radula, *e.g.*, *Tethys*, may at one time be carnivorous, at another time phytivorous; likewise forms possessing radula (*Acanthodoris*) are omnivorous. The largest number seem to be carnivorous notwithstanding; a few, the *Hermæidæ* and *Elysiidæ*, are phytivorous.

Means of Defense

Upon being first encountered, *Melibe leonina* appears brown, but when examined in the aquarium one can easily see that the brown coloring is rather superficial in comparison to the marked transparency which the body possesses. This transparency is so great that the internal organs, such as the alimentary canal, the organs of reproduction, and the heart, can be easily seen. The possibility of actually seeing these organs through the body wall is due to the arrangement of the muscles and the connective tissues, and because the body-fluid contained in the perivisceral cavity, between the visceral

organs and the muscles, the connective tissues, as well as the blood, are colorless. This characteristic is also common to *M. pellucida* Bergh, 1904; and *M. vexillifera* Bergh, 1880.

Upon touching the curious-looking animal it gives off a peculiar odor. This is rather strong, and resembles that of oil of bergamot. It is caused by a secretion from small compound saccular glands lying immediately under the ectoderm (Fig. 12). These glands are distributed all over in the external parts of the body: in the body wall, the papillæ, under the ectoderm of the exterior part of the hood, and in some cases, under the ectoderm of the foot. None of these glands seem to be present under the ectoderm of the ventral side of the hood. The extent of distribution of the odoriferous glands seems to indicate that they have a definite use and purpose, *e.g.*, that of defense.

Meckelii, 1838, describes the odor exuded by *Tethys leporina* as resembling citron, or being rather pleasant. And Bergh, 1877, says:

> Von . . . toten Thiere habe ich irgend eine Spur bemerkt, dagegen einen nicht starken, etwas besonderen, aber nicht wesentlich unbehablichen Gestank.

Hecht, 1895, discusses the various means of defense possessed by Nudibranchs. He mentions protective coloration, nematocysts, mucous glands and death feigning. The last will be discussed presently; the first two may only be referred to, as *M. leonina* possesses no protective coloration, and has no nematocysts as a defensive means. It may, however, be stated that in the aquarium, *Melibe leonina* became even more transparent than it was when seen in its natural environment. This change of color was also observed by Alder and Hancock, 1845, on a number of Nudibranchs kept in captivity: "In such cases they generally lose a good deal of colour and become very transparent," and that coloration is not caused by the color of the food taken, but by the color of the liver and gonads. Eliot, 1910, says:

The colour of Dorids is to some extent affected by their food, though less than that of Eolids. The brightly coloured species often frequent and feed on similarly bright sponges or Ascidians, and when they do not obtain their usual food in confinement they lose their colour (p. 5).

An increased transparency when kept in an aquarium, *e.g.*, a glass-jar, may be designated adaptive coloration. The odorous substance that the animal exudes when touched by an enemy is its main protection. However, Step, 1901, records some very interesting facts relative to protective coloration among Nudibranchs:

Dendronotus frondosus is obviously adapted to life among sea-weeds and coralline, resembling some small red-brown sea-weeds (*Collithamnium*). It is said to be highly edible, having nothing in its flavor to displease the taste of the most fussy fish; and therefore its disguise is absolutely necessary to the species (p. 288–289).

Evidently *Melibe leonina* needs no protective coloration, having, as said above, odoriferous glands. One reason why it does not possess nematocysts is perhaps because it does not live on Hydroids, but mostly on Crustacea.

Glaser, 1903, in his discussion of the origin of nematocysts in Nudibranchs gives a historical review, including a number of citations all of which refer to the nematocysts as having been taken in with food. The food then seems to be the origin of the nematocysts in the Nudibranchs; *M. leonina*, which does not feed on hydroids, has no nematocysts in its system. The marble slug, *Lomanotus marmoratus*, according to Step, "Feeds upon Corallines which it closely resembles in colour and ornamentations"; Gamble, 1892, however, says that enidocysts are absent.

Bergh, 1890*a*, mentions experiments by Krugenberg, 1880, who tried to determine the physico-chemical constituents of the odoriferous glands, as well as of the liver and blood of *Tethys fimbriata* (s. *leporina*), and says that *T. leporina* has a peculiar musk-like nauseous odor which it uses as a means of defense against its enemies. This is, without doubt, the office of the odor in the case of *M. leonina* also. The actual nature or constituents of

the substance which causes this defensive odor have not been determined.

Another means of protection is self-mutilation, exemplified by *Discodoris fragilis*, that according to Eliot, 1899, throws off part or the whole of its mantle edge. Collingwood, 1868, also records this habit of self-mutilation of a *Doris*.

EMBRYOLOGY

The Egg-body (Nidosome)

Bergh, 1902, describing the egg-body of *Melibe bucephala* says:

> The spawn forms a large heap of a diameter of 3.5 cm., composed of the innumerable windings of a dull yellow tube of a diameter 0.75 mm. The tube contains inside of the tough transparent covering several series of displaced, more or less cleft eggs.

From this description it is clear that this nidosome is quite different from that of *M. leonina;* it seems, indeed, strange that the egg-body of two closely related species can differ so widely. The external features of *M. bucephala,* according to Bergh's description, are not much different from those of *M. leonina.* It may be a question whether the egg-body attributed to *M. bucephala,* by Bergh, actually belongs to this species.

The writer during the summer of 1914 found several nidosomes among the eel-grass, but it was not known to which animal they belonged until *Melibe leonina* was seen to lay the same kind in the aquarium of the laboratory (Agersborg, 1919). These nidosomes of transparent mucous, or gelatinous substance, were funnel-shaped, when suspended in the water, with the apex attached to some solid object (Fig. 3). The average slant-height of these conical structures was 5 cm., with a perimeter of about 28.2 cm. and a convex surface, therefore, of about 70.50 sq. cm. From the adhering point of the nidosome, dotted lines, the capsules radiated to the periphery of the conical body. This radiation was not so regular in

some as in others, yet there was a prevailing regularity in this respect. In Fig. 3, the arrangement of the capsules does not represent the prevailing regularity, as it was necessary to select an entire nidosome for photographing, most of the other having been broken. The capsules contained from 10 to 22 eggs (Figs. 4–6). The actual method of deposition has not been observed, but

FIG. 3. Photograph of a nidosome of *M. leonina*, natural size. The dark band at right angle to the fold is a piece of eel-grass to which the egg-body is attached. The many radiating white dots are egg-capsules containing from 15–22 eggs.

it is conjectured from the knowledge of the anatomy of the animal that the capsules are imbedded in the gelatinous mass as the nidosome is deposited. The mucous gland, which consists of (1) albuminous gland, (2) nidamental gland (Lang, 1896), is in *Melibe* in direct connec-

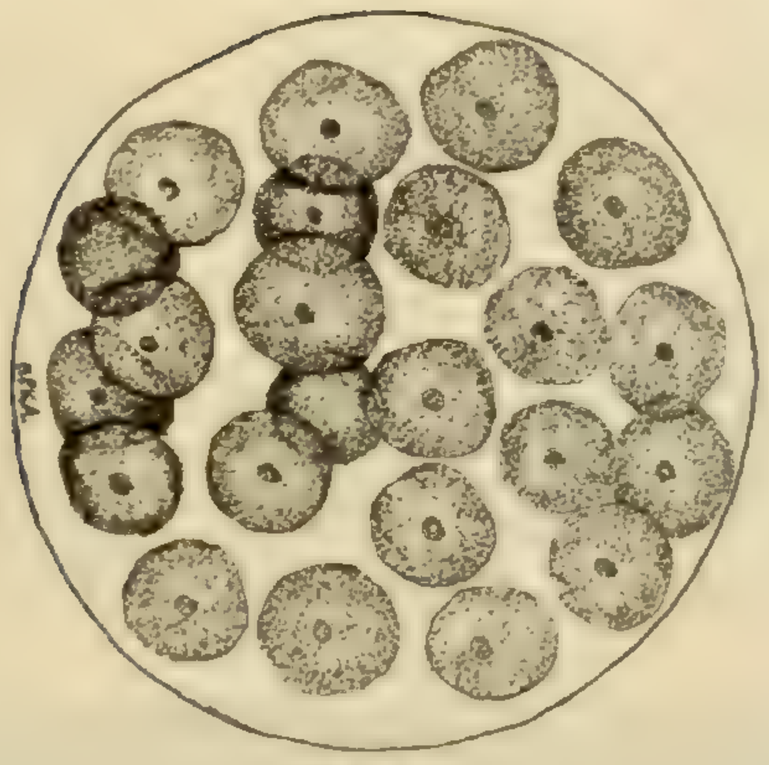

Fig. 4.

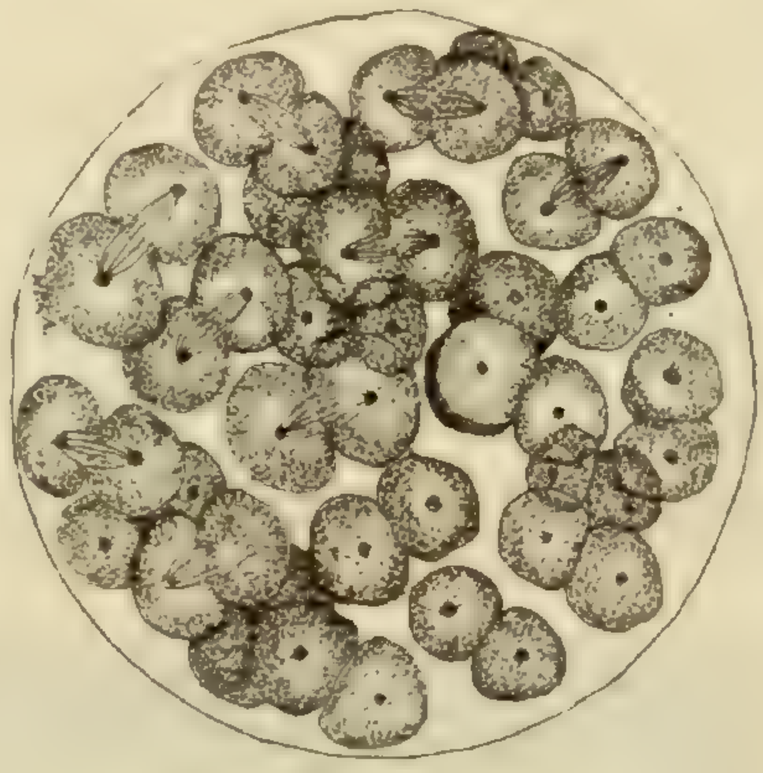

Fig. 5.

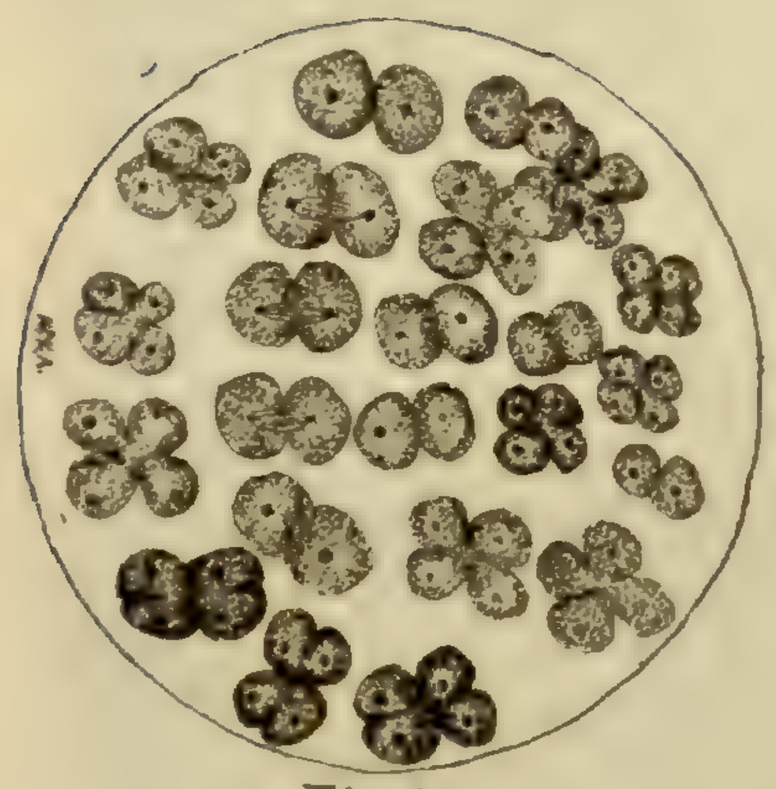

Fig. 6.

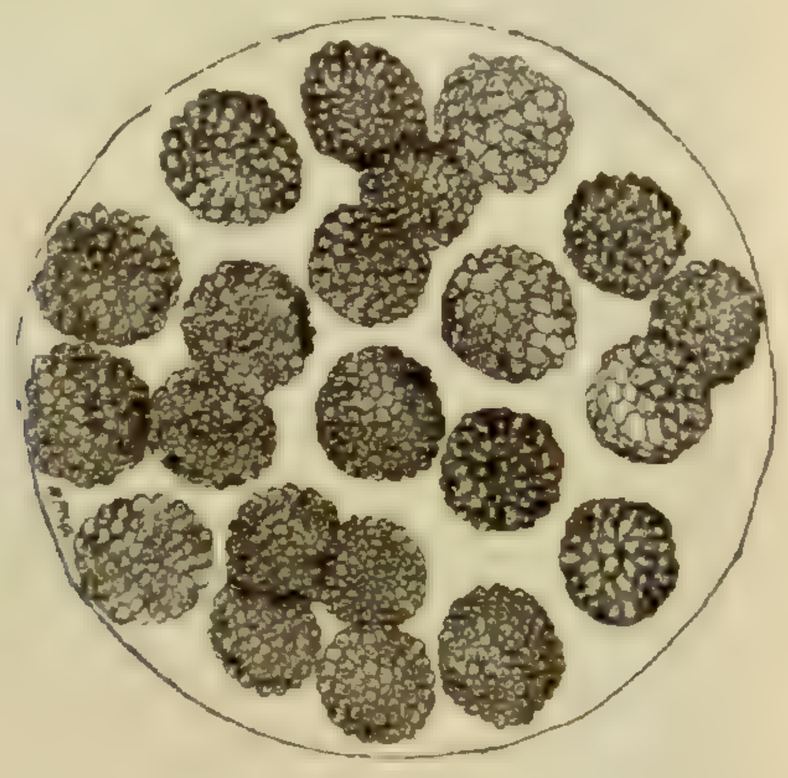

Fig. 7.

FIGS. 4–7. Drawing of egg-capsules from a nidosome laid in captivity, showing a varying number of eggs. Figs. 5–6 show the eggs in two and four cell stage, three hours after the nidosome was laid. In Fig. 7 cleavage has reached the blastula stage. The more oblong embryos are moving within the capsules.

tion with the vaginal orifice. In copulation, the penis, which is long, twisted like a screw, and of tough musculature, is inserted into the posterior genital pore of the mate, and so firm is the union that separation may not occur even though the couple be dipped from their natural abode and placed in a vessel.

Observations in the Laboratory

A study of *Melibe leonina* from an embryological standpoint, was made at the Biological Station. From the lot collected, some were preserved, others were kept alive in an aquarium. One morning, however, all save one were

dead. Later, this one also seemed dead, and it was thought that the water had become stale. *Melibe* lay absolutely motionless on the bottom, all its muscles completely relaxed, and showed signs of life only after the water had been oxygenated for several minutes. It is muscular relaxation of this sort that *Melibe* assumes when it sinks from the surface to deeper water. After this the writer became used to its death feigning and needed only to oxygenate the water for it to become active again, crawl along the bottom and side of the aquarium, and after a while start swimming in the vessel. Changing of the water could not be done indefinitely, as on another morning a nidosome was found to have been deposited by the animal during the night. It was a funnel-shaped, transparent, gelatinous body adhering by the tapering end to the side of the jar. Viguier, 1898, when trying to prepare a specimen of *Tethys fimbriata* for fixation, observed the same phenomenon. He, however, did not change or oxygenate the water; he left the animal in it for about two weeks, when he found that an egg-body had been deposited, the eggs having ceased to divide in the four-cell stage. The nidosome of *T. fimbriata* is quite different in shape from that of *M. leonina*. In the case of *Melibe* the eggs continued to divide until they were transformed into larval forms, which actually turned the whole nidosome into a vibrating mass. The development continued, apparently normally, until the larvæ left the capsules, when they soon died. That the delicate molluskan young should die when coming in direct contact with the water of the aquarium was expected, as the renewing of the water was stopped after the nidosome was deposited, it being thought undesirable to disturb it too much; after the deposition of the egg-mass the water was simply kept at constant level, and oxygenated from day to day, so that the animal should not die. When the young *Melibes* were hatched the mother animal, without being further inseminated, laid another nidosome, which also hatched two weeks later. The eggs of the first nido-

some developed into distinctly living creatures, moving about in the capsule on the fifth day after the setting (Fig. 7); it took two weeks for the complete development of the young. Alder and Hancock, 1845, p. 25, say:

> The embryo matures after deposition of spawn, from a few days to a month or more, according to species; the actual time appears to be about ten days or a fortnight.

Temperature, no doubt, plays an important part in the speed of development. Stuart, 1865, says:

> Gewöhnlich wird von den Opisthobranchiereiern angegeben, dass die Dauer der Entwickelung des Embryo ein Monat ist, in meinem Falle war sie circa zwei Monate; dabei war die vorherrschende Witterung, die für Sicilien jedenfalls eine kalte zu nennen war, gewiss von grossem Einflusse (p. 96).

The second nidosome was to some extent abnormal, compared with the first, and with those collected from the eelgrass. It showed a variation of the number of eggs in the capsules, from one to fifteen (Figs. 8–9). This abnormality was perhaps an indication of the decline in vitality of the mother animal. In fact, the adult specimen had greatly decreased in size since its capture.

One difficulty was that of keeping the water at a constant density. In order not to break the nidosome the water was only oxygenated and kept at a constant level. Each time when the eggs were examined, small portions of the nidosome were removed, and by so doing the membrane of the egg-body was broken. This did not seem to affect the development, however. Yet it was thought safer to keep the water at the same temperature as hitherto, than to change it daily, as the latter might cause too great physical shock. The abnormality of the water, as said, did not affect the embryos as long as they were within the capsules of the nidosome. Perhaps thus far in their development they were not affected by the abnormality of the water; even though the egg-body was punctured and broken in the examination of the eggs, the embryos seemed all to develop, as far as could be detected,

and to pass through the normal developmental changes of a typical gasteropod.

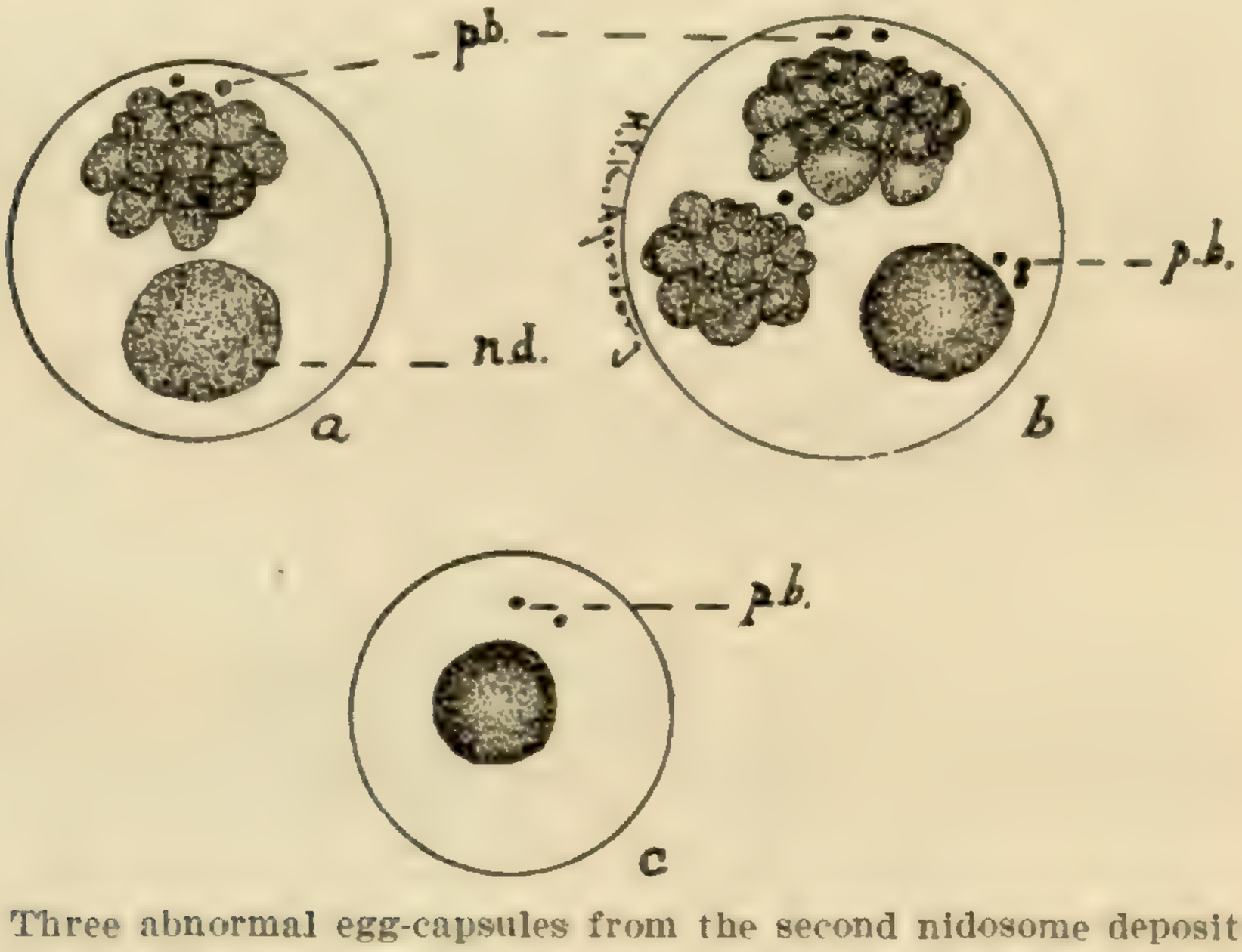

FIG. 8. Three abnormal egg-capsules from the second nidosome deposited after insemination; *a, nd.* no cleavage. *pb,* polar body; *b, pb.* first polar body has divided, but no cleavage in the egg; *c,* no cleavage of egg although the polar bodies were given off.

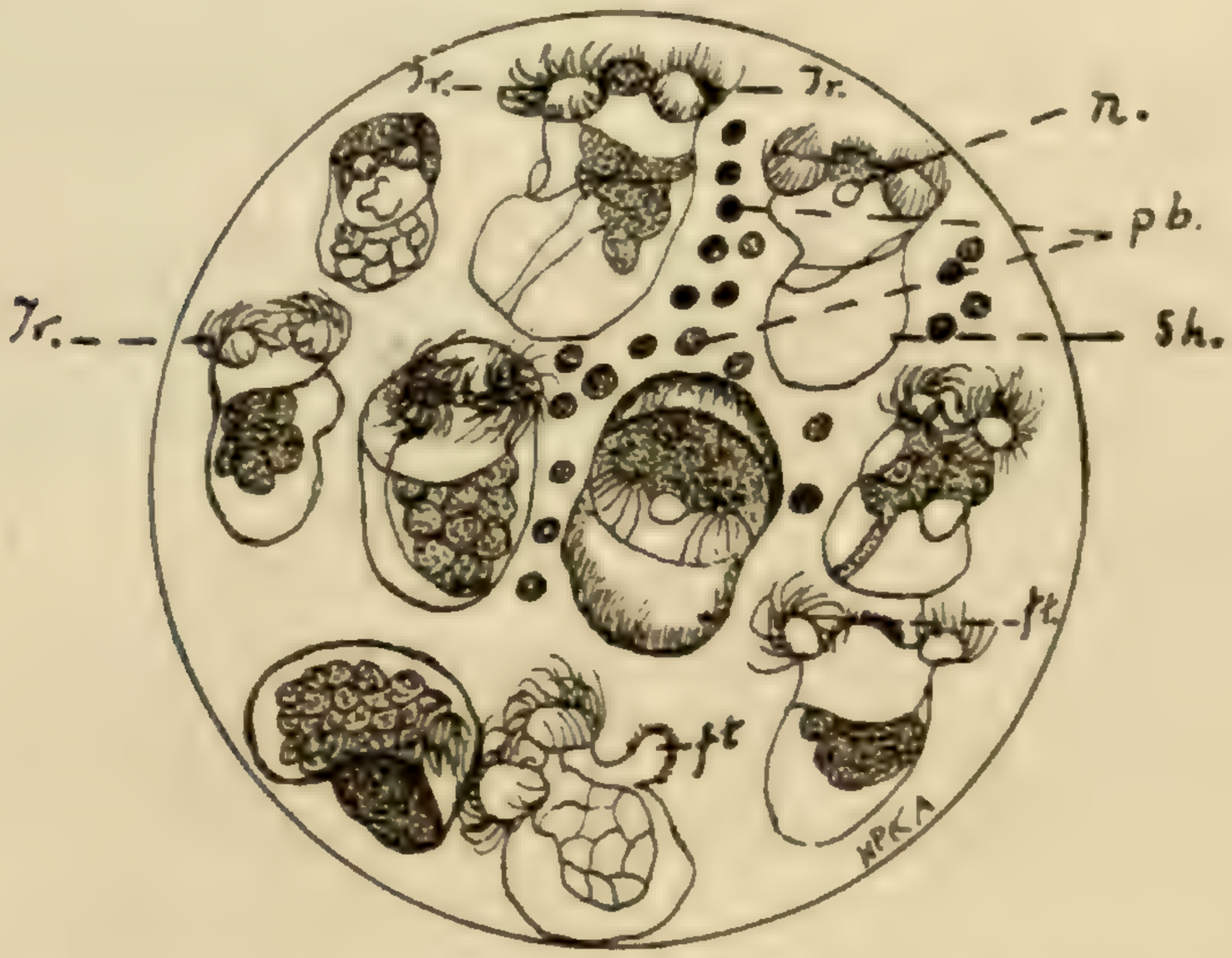

FIG. 9. An average sized capsule from the second setting after insemination; *n,* pedal ganglion; *ft,* foot; *pb,* polar body; *sh,* shell; *tr,* velum; all the embryos are in the veliger stage.

Abnormality in the second nidosome was marked by the reduced number of eggs within the capsules and in the early development in the eggs. The variation of the number of eggs in normal capsules was most marked at

the end of the egg-belt. This abnormality was much greater in the second nidosome, in that there was a much greater number of small capsules with only one or a few eggs in them. There were also capsules actually without eggs. Some eggs failed to develop; some gave off the polar bodies and then did not advance any farther; others did not form polar bodies. It was of great interest, indeed, to watch the development of the embryos in the large capsules which in some cases contained more than twenty eggs (Fig. 7). Within a few hours, there would be twice or even three times as many polar bodies as eggs (of course within the capsules), because the first polar body sometimes divided. A detailed study of the blastomeres was not undertaken. Figures 4 to 6 show a few early developmental stages, and early and late larval stages are shown in Figs. 7 and 9. An embryonic shell is shown in Fig. 10; veliger larvæ in Fig. 9.

When the embryos reached the gastrula stage they swam about within the capsules. On the fifth day after being laid the whole egg-body was practically alive with imprisoned swimming larvæ (Fig. 9). Nine days later, the larvæ began to leave the capsules. It was surprising to see how rapidly the embryos advanced from day to day, going through the trochophore and veliger stages. During the latter stage the shell was very prominent; it resembled the shell of *Natica russa*, in that it had a blunt apex and short body; the posterior or tapering part of the shell had no spiral turns; the posterior edge of the aperture had a small indent; the edge of the aperture of the shell was otherwise without any modifications (Fig. 10). The animal itself did not assume the adult shape before it lost the shell, but when it left the capsule it shed the shell, and the young began the life of a so-called naked mollusk. The presence of a shell with operculum in embryonic life of Nudibranchs has been observed by various authors: Alder and Hancock, 1845; Pelseneer, 1893; Smith, Bell and Kirkpatrick, 1905; Boas, 1916, and others.

A large number of eggs were present in each capsule of the first nidosome (Figs. 4–7), and all developed into

FIG. 10. Embryonic shell of *Melibe leonina*.

embryos which finally went through metamorphosis. In the second nidosome, some of the eggs failed to develop. The cause was perhaps lack of spermatozoa. During copulation, the spermatozoa become stored in the spermatotheca but also wander up the uterus as far as the prostate gland (Fig. 11, *spt.*, *ovd.*, *pr.*). The eggs are probably fertilized while passing down the uterus, *ut.*, or while in the spermatotheca, *spt.*, as many eggs actually pass into this out-pocketing of the uterus, the spermatotheca may therefore be termed ovo-spermatotheca. The only means of regulating the fertilization process in the egg must be the physical condition of the egg, which determines the reception or the function of the sperm; as all eggs, under natural conditions, contained in one and the same capsule, and, indeed, in the entire nidosome, go through simultaneous development, although they all (perhaps more than 100,000, in one normal deposit, nidosome) can not possibly have been fertilized at the same moment. This primary part of fertilization must take place before the eggs are incapsulated. If, however, an insufficient number of spermatozoa are present during the flow of the eggs, some eggs may become incapsulated without having been fertilized. One thing noted was

that the eggs in one end of the nidosomic belt, and in the main part of it, were all fertilized, while the other end (the last end) of the belt showed lack of fertilization. Another fact noted is that *Melibe leonina* carries over spermatozoa in its genital vessels; that more than one egg-body is deposited after insemination.

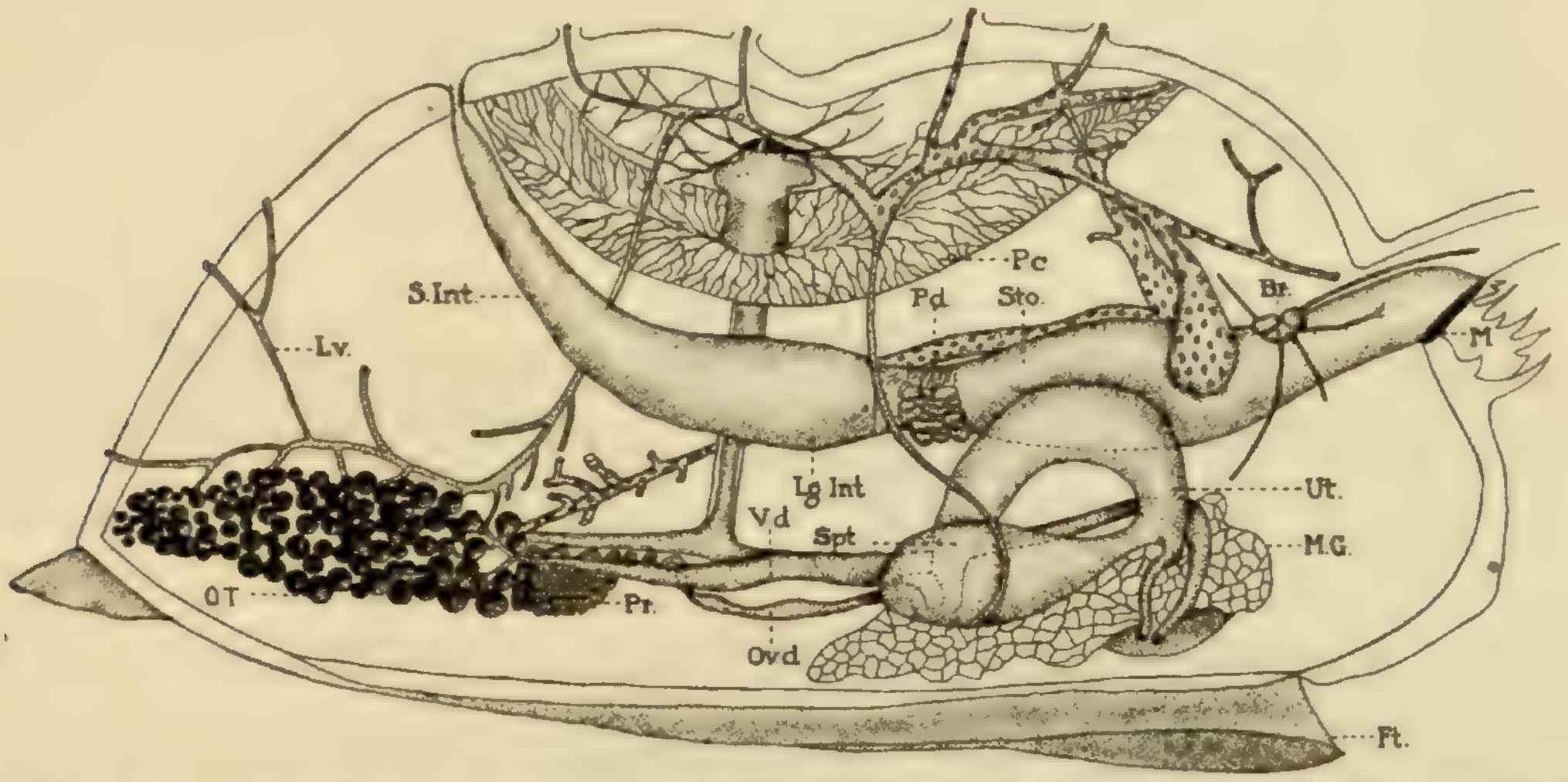

FIG. 11. Schematic drawing of dissected adult animal to show the general arrangement of the visceral organs: *Br*, brains; *Ft*, foot; *Lg. int*, anterior and large part of the intestine; *M*, mouth; *Mg*, mucous gland; *Or. d*, oviduct; *Pc*, pericardium; *Pr*, prostate; *Si*, small part of the intestine; *Sto*, stomach; *Ut*, uterus; *Vd*, vas deferens; *Lv*, branch of liver; *Spt*, ovo-spermatotheca.

Reid's observations, 1846, on *Doris bilamellata, D. tuberculata, Gonidoris barvicensis, Polycera quadrilineata, Dendronotus arborescens, Doto coronata,* and a species of *Eolis,* bring out the same fact, viz., more than one deposit takes place after insemination. From 26 hours after coitus deposition may begin; "it does not, however, appear to be absolutely necessary for the production of fertile ova in all, if in any of the individuals of the nudibranchiate Mollusca, that coitus should have so shortly preceded spawning as was observed in Polycera, for an Eolis which was kept strictly confined in a vessel by itself, deposited, on the tenth and again on the thirty-second day of its isolation, abundance of fertile ova." Crozier, 1919, claims that the larger animals of *Chromodoris zebra* Heilprin, lay several more egg-masses in a given time than do small ones; that it is consequently of advantage to the species that large individuals

should mate together; that there is, in fact, selective pairing which is of a distinctly advantageous or "purposeful" character, since it makes for the multiplication of the species. The same author, 1917*a*, records the unique findings relative to a rather high degree of correlation between the sizes of the two pairing members of *Chromodoris zebra*. The writer has observed the same fact relative to copulation among the Eolidæ. As for *Melibe*

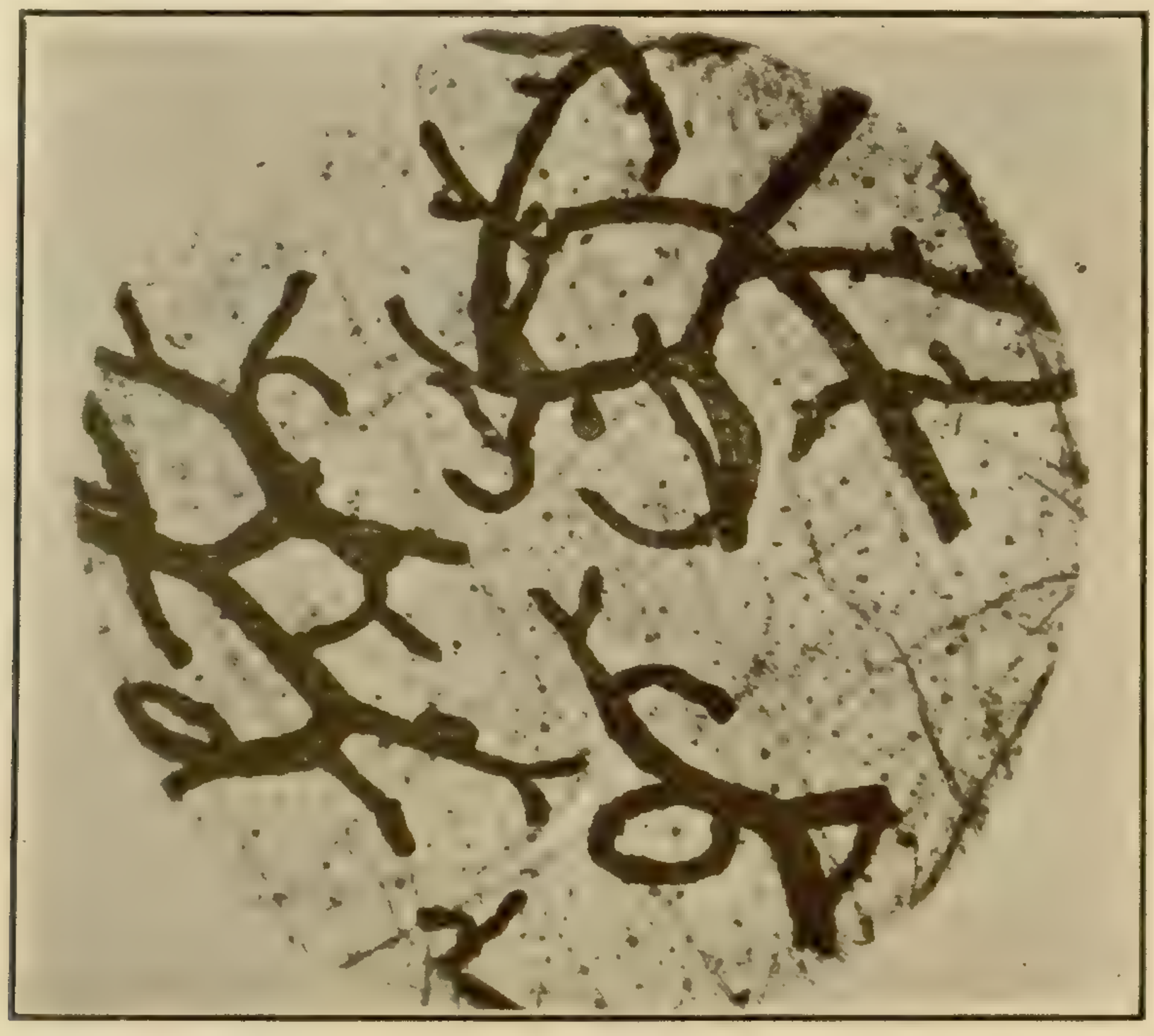

FIG. 12. Micro-photograph of the inner side of the ectoderm of the body-wall, showing the odoriferous glands, the small dots among the branching (black) hepatic cæca, and the crossing muscle (pale) fibers.

leonina, it is also true that those found copulating were of the same relative size. But even so, as seen in the second nidosome of *Melibe*, the animal may run short of spermatozoa during ova-deposition. To guard against this, there is, as shown by Crozier, among certain species, selective pairing between individuals of nearly the same size. Garstang, 1890, finds a considerable variation between the offspring of *Lomanotus* Vérany, "the individuals apparently showing a tendency to unite rather with those of their own variety than with those unlike themselves."

To understand the process of insemination of the eggs, and the conditions controlling the number of eggs in the capsule, a little speculation is necessary. It seems as if the processes of fertilization and incapsulation are effected during the emission of the eggs; that when the eggs pass down the uterus or pass the spermatotheca they are fertilized, and immediately after that incapsulated, and that the size of the capsules and the number of eggs present in each capsule are regulated by the speed of the outflow of the eggs. The size of the capsule, as a rule, varies according to the number of the eggs present within the capsule. It seems puzzling, however, when capsules are found without eggs, and with eggs which show no indication of being fertilized, but this abnormality is limited to the last part of the nidosomic belt, and is of course so controlled that an entire egg-body may not be deposited without some eggs at least being present, and being fertilized. It is a matter for future observation to determine whether individuals in ova-maturity are capable of depositing normal nidosomes, without being stimulated by an individual in ripe male-phase. It is a question whether the mere pressure of ripening eggs will cause egg-flow. Crozier, 1917*b*, reports, however, that *Chromodoris zebra*, if left alone, deposits fragments of egg-bodies which are not fertilized. The writer has noted the same phenomenon relative to *Eolis olivacea* when it is kept alone; but also in this case, as in the case of *Chromodoris*, no normal nidosomes were deposited.

The question of cross-fertilization becomes of interest since spermatozoa are found in both genital ducts of the same individual, from the ampulla of the penis and all the way down the penis to the end of it; from and including the prostate which surrounds the uterus, to and including the ovo-spermatotheca. If self-fertilization takes place, should there be any shortage of spermatozoa during ova-deposition? The presence of spermatozoa in the female genital tube is undoubtedly the result of coition and the

wandering of the spermatozoa up the uterus, against the outward current of that organ. Alder and Hancock, 1845, p. 25, say:

> The Nudibranchs, notwithstanding that they are androgynous, frequently copulate during the breeding season. The conjoined individuals lie side by side, their heads turned in opposite directions. Thus the right sides of the two animals are brought in close contact, and mutual impregnation is effected. They remain in this position for some time, but in a short period after separating, generally about the first or second day, the spawn is deposited.

Crozier, 1919, claims that *Chromodoris zebra* is functionally hermaphroditic, and effective reciprocal insemination is practised. But this is not practised among the species *Melibe leonina;* although semen may be present at the same time in both genital ducts, insemination is not reciprocated simultaneously. That is, in all the individuals examined, coitus was effected by the introduction of the penis of the one mate; the penis of the other mate was completely withdrawn. Whether spermatozoa were present in the members whose external genital organs were not visible was not determined. It looks, however, as if *Melibe leonina* is protandric, a condition, according to Pelseneer, 1895, common among *Eolis, Elysia,* and *Clione limocina.* Eliot, 1910, says:

> Pairing, according to Hecht, is reciprocal, and though hermaphrodite Mollusca are incapable of self-impregnation both individuals spawn after mating.

The writer has observed on *Eolis olivacea,* at Woods Hole, that one mate may start spawning while copulating. Spermatozoa, according to Reid, may be carried in the female genitals (*Eolis*) for more than thirty days before being used. That is, cleavage does not start in the eggs of *Eolis* until after deposition; fertilization, therefore, may not occur before the time of incapsulation. Spermatozoa are kept alive in, and stimulated by, secretions of the female genital organs, as shown by Eliot and Evans, 1908, p. 287:

The walls of the spermatotheca (of *Doridoides gardineri*) are thick and produce a secretion. In some specimens small clumps of spermatoza are embedded in this secretion. In others all the spermatozoa form a central mass in the main cavity of the spermatotheca. It is possible that the secretion serves to form small packets of spermatozoa or spermatophores.

SUMMARY

1. *Melibe leonina* is a large carnivorous Nudibranch reaching sometimes 14 centimeters in total length; it is an actively predaceous animal; it practically gorges itself, feeding mainly on small Crustacea; it is gregarious.

2. It seems to live more than one year; its recurrence is spasmodic.

3. It swims freely in the water, backward, upward or downward; it crawls on the surface by the surface tension, and on sea-weeds, by the help of its highly ciliated foot.

4. Its defensive means are an offensive odor and death feigning.

5. It drops to deeper water by relaxation of its muscles.

6. It collects in groups among sea-weeds, where copulation takes place.

7. Mutual insemination does not seem to be simultaneous.

8. It spawns as early as March and as late as July; sexual maturity is reached quite early, as young ones two centimeters long were found with ripe spermatozoa.

9. Spermatozoa from another individual are stored in the ovo-spermatotheca but wander up the uterus as far as the prostate.

10. Eggs are also stored in the spermatotheca, hence the name ovo-spermatotheca.

11. Copulating individuals are of the same relative size.

12. The same individual deposits more than one nidosome, after insemination; spermatozoa may be carried over in the ovo-spermatotheca at least two weeks.

13. The eggs are deposited in capsules, normally containing from 15 to 22 eggs. The capsules are arranged in rows within a gelatinous mass, sometimes quite regularly; the gelatinous mass is formed into a belt from 3 to 5 cm. wide; the mucous flow is greater in one side of the belt than in the other, so that one side of the belt is shorter than the other, and the belt curves into a funnel-shaped mass, the apex adhering to some sea-weed, near the surface of the water.

14. Eggs may become incapsulated without being fertilized; no cleavage of such eggs follows.

15. Normally the embryo develops within two weeks.

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TYPES OF MUTATIONS AND THEIR POSSIBLE SIGNIFICANCE IN EVOLUTION.[1]

DR. ALBERT F. BLAKESLEE

STATION FOR EXPERIMENTAL EVOLUTION

THE beginning of the twentieth century saw the rise of two concepts which have profoundly affected biological thought and been of increasing influence in the trend of experimental study of plants and animals. The mutation theory of deVries based on the evening primrose, and the laws of Mendel based on the garden pea, settled the date of birth of the modern science of genetics. The studies on these two plants have together formed a basis for the main bulk of our present genetic investigations. While the garden pea stands intimately associated with a conception of inheritance of wider application than was at first imagined, the evening primrose and the theory of mutation connected with it are by many considered to furnish an example of a valuable theory founded upon incorrect interpretations. The belief is growing that most of the new forms which have appeared in cultures of the Œnotheras are not mutations at all and that the evening primroses, as an abnormal group of plants, are not to be seriously considered as representative of the processes of evolution in normal forms.

In the short time at my disposal, I wish to outline some recent findings in the jimson weed (*Datura Stramonium*) which it is hoped may throw incidentally some light on the more highly involved phenomena in the Œnotheras, and which may serve as a basis of a brief discussion of their possible evolutionary significance.

The jimson weed is not supplied with a wide range of obvious Mendelian characters. The early studies of

[1] A paper presented before the American Society of Naturalists at the Chicago meeting, December 30, 1920.

Naudin (9) and Godron (7) as well as the later investigations of deVries (13), Bateson and Saunders (1) and the writer and Avery (4) on this species have shown that purple color in flower and stem is dominant to lack of purple in those parts, and that spiny capsules are dominant to smooth capsules. The writer with Avery (5) has been able to add a third pair of contrasting characters: "many nodes," causing tall stature, in contrast to "few nodes," causing low stature. These are all the allelomorphic pairs actually determined. Moreover, no variation has arisen in the writer's cultures during the last seven years' study of this species which gave evidence of differing from the present stock by a single Mendelian factor. Distinct variations, provisionally termed mutations, have, however, regularly recurred whenever a sufficiently large number of plants have been subjected to observation (5). So far as investigated, they have been found to be connected with a duplication of one or more of the normal chromosomes (6). The normal quota is 12 pairs: 12 being therefore the gametic, haploid, or x number; and 24 the somatic, diploid, or 2x number. The simplest type of duplication is the addition of an extra chromosome, probably by non-disjunction in one of the pairs of the diploid complement, giving $24+1$, or 25 chromosomes as the somatic number. In such plants there will be 11 sets of 2 homologous chromosomes each and 1 set of 3 homologous chromosomes.

We have on the chart, Table I, 12 recurrent mutants of the type just discussed; which, while perfectly distinct from each other and from the normal stock, have certain characteristics in common. All have been found to produce gametes with 12 *and* 13 chromosomes (therefore with 25 as the calculated somatic number); all have a relatively large proportion of bad pollen grains, varying in the different mutants from 8 per cent. in the Globe to 21 per cent. in the Spinach, as indicated in the chart; all fail to transmit the mutant complex to any considerable extent through the pollen, while they do

TABLE I

SOMATIC NUMBER OF CHROMOSOMES AND PERCENTAGE OF BAD POLLEN FOUND IN NORMALS AND IN DIFFERENT MUTANTS

Types	Somatic Number of Chromosomes	Per Cent. Bad Pollen
NORMALS	24	2.7
MUTANTS		
Tetraploid (''New Species'')	48	3.3
Triploid	36	34.1
Simple Trisomic		
1. Globe	25	7.9
2. Poinsettia	25	12.9
2a. P. var. wiry	25	9.3
3. Cocklebur	25	18.3
4. Ilex	25	12.2
5. Mutilated	25	20.7
6. Sugar loaf	25	16.1
7. Rolled	25	8.4
8. Reduced	25	10.7
9. Buckling	25	10.4
10. Glossy	25	18.0
11. Microcarpic	25	12.8
12. Spinach	25	20.7

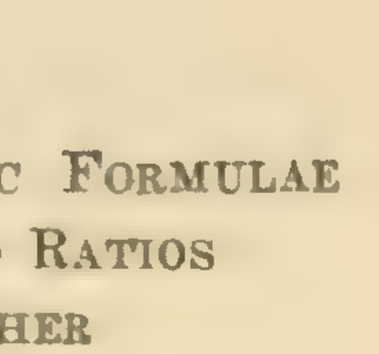
transmit it through the egg cells, although to only about one quarter of the offspring. That the offspring of these mutants repeat the parental type regularly in less than the 50 per cent. expected is probably due to the lessened vigor of growth of mutants in comparison with normals.

If the presence of an extra chromosome in a given set causes a specific mutation due to the constitution of this particular chromosome, rather than to the mere presence of an extra chromosome irrespective of its origin, there are at least two consequences to be expected. First there should be as many possible mutants of this type as there are chromosome sets which may undergo duplication. In other words there should be 12. Twelve, as a matter of fact, is the actual number which we had found before the nuclear condition had been determined. In addition, we have two or three mutant forms apparently belonging to this class for which it has not yet been possible to obtain chromosome counts. In appearance they are combinations or modifications of members of the

recurrent twelve. Secondly, it should be possible by breeding tests to connect up mutants with as many chromosome sets as there are known Mendelian factors, or factor groups. This connection we seem to have established between the mutant Poinsettia and the set of chromosomes which carries the factors for purple pigmentation in flower and stem.

TABLE II

TYPES OF CHROMOSOMAL DUPLICATION, GAMETIC AND SOMATIC FORMULAE FOR PLANTS HETEROZYGOUS FOR FACTOR PAIR A AND a AND RATIOS OBTAINED WHEN SUCH PLANTS ARE SELFED, TOGETHER WITH DIAGRAMS ILLUSTRATING THE CHROMOSOMAL CONDITION IN SOMATIC CELLS

No. of Extra Chromosomes in Set	No. of Sets Affected	Gametic Formula	Selfed Ratios	Somatic Formula	Somatic Diagram
2	12	AA + Aa AA + 4Aa + aa Aa + aa (12 + 12)	1A : 0a 35A : 1a 3A : 1a	AAAa AAaa Aaaa (12 + 12) + (12 + 12)	
1	1	2A + a + AA + 2Aa A + 2a + 2Aa + aa 12, (12 + 1)	NOR. 8A : 1a MUT. 9A : 0a NOR. 5A : 4a MUT. 7A : 2a	} AAa } Aaa (12 + 12) + 1	
1	12			(12 + 12) + 12	

No. of chromosomes 12 +	0	1	2	3	4	5	6	7	8	9	10	11	12
Frequencies	1	12	66	220	495	792	924	792	495	220	66	12	1

The set of 3 chromosomes in the diagram, Table II, may be called the Poinsettia set, or the purple set. A Poinsettia plant may, to speak in terms of the dominant factor, be considered nulliplex with no dominant genes, or simplex, duplex or triplex with, respectively, 1, 2, or 3 dominant factors. There are therefore two types of heterozygotes, and under greenhouse conditions these apparently can be distinguished from each other as well as

from the homozygous dominants by different intensities of pigmentation. Simplex heterozygotes when selfed throw offspring with 5 dominants to 4 recessives among the normals, and 7 dominants to 2 recessives among the Poinsettias; while duplex heterozygotes should give a ratio of 8:1 among the normals, and all dominants among the Poinsettias. There is evidence which seems to indicate that the mutant Cocklebur is conditioned by duplication in the chromosome set which carries both the factors for spiny capsules and also those for number of nodes. If this is actually the case, we must assume that these two factor pairs are loosely linked in the same chromosome with about 50 per cent. crossing over, since they appear to segregate independently of each other.

We have been discussing duplication of a single member in only one of the 12 chromosome sets. On the lower part of the chart (Table II) is represented the only plant we have yet found with an extra chromosome in every one of its 12 sets. Such a plant is triploid. What its breeding behavior will be, can not be told before another season. If the chromosomes assort at random, the gametes theoretically should have the chromosome numbers indicated in the chart, and the counts which my colleague, Mr. Belling, has made from figures in pollen mother cells are not inconsistent with the distribution of the theoretical frequencies. One might expect such triploid plants to give rise to individuals intermediate between triploids and mutants of the Poinsettia type; in other words to mutants with duplication of chromosomes in 2, 3, 4, etc., up to duplication in all the 12 sets. Such compound mutants we have not yet been able to surely identify in our cultures; but we have never before this past season had a triploid plant, which from the wide range of gametic types in its egg cells would seem a likely source of such mutations.

Tetraploid plants have been discussed at yesterday's session of the Botanical Society of America. They represent a further duplication over those of the triploids

already mentioned in that there are 4 homologous chromosomes in each set in somatic cells. The homologous chromosomes therefore form tetrasomes, to use a new term,[2] instead of disomes as in normals or trisomes as in triploid plants. Members of these tetrasomes apparently assort at random in the reduction division. In consequence, certain peculiarities in breeding behavior result. Plants duplex for a dominant factor (AAaa) will, when selfed, give a ratio of 35 dominants to 1 recessive in the offspring. Plants simplex for the dominant (Aaaa) will give a 3:1 ratio in their offspring; but a third of the dominant offspring will throw 35:1 ratios in the next generation. Plants triplex for the dominant (AAAa) will give in the immediate offspring all dominants; one quarter of which, however, may be expected to give a 35:1 ratio in a later generation. The results expected from selfing the 5 zygotic types are shown in Table III.

It might be expected that mutant forms would be found in which doubling of the chromosomal number had involved only a single one of the 12 sets. Such mutants would bear the same relation to tetraploid plants with all the sets involved that the Poinsettia type of mutants bear to triploid plants. They have not yet been found, however.

[2] The following terms are suggested to designate sets with numbers of chromosomes from 1 to 12: monosome, disome, trisome, tetrasome, pentasome, hexasome, heptasome, oktasome, enneasome, dekasome, hendekasome, dodekasome.

The number of sets affected by duplication may be indicated by the terms: simple, double, triple, quadruple, quintuple, sextuple, septuple. octuple, nonuple, decuple, undecuple, duodecuple.

The Poinsettia and Globe are simple trisomic mutants. If the Globe and Poinsettia could be combined to form a mutant with 3 chromosomes each in two of the 12 sets, such a mutant would be called a double trisomic mutant. If differential viability of gametes does not interfere, the triploid plant already mentioned should produce, theoretically, offspring of all the trisomic types from simple to duodecuple. Haploid, diploid, triploid, tetraploid, etc., are terms already employed to designate plants with the same number of chromosomes in all the sets.

TABLE III

TETRAPLOID PLANTS. RESULTS OF SELFING TETRAPLOID PLANTS ARISING FROM THE CROSS OF A HOMOZYGOUS DOMINANT (AAAA) BY A RECESSIVE (aaaa), CARRIED TO THE F_4 GENERATION

In the F_4, only phenotypes are represented.

P_1— AAAA and aaaa

F_1—AAaa (Gametes of F_1 — AA + 4Aa + aa)

F_2—1AAAA + 8AAAa + 18AAaa + 8Aaaa + 1aaaa

F_3—AAAA AAAA + 2AAAa + AAaa AAaa + 2Aaaa + aaaa aaaa

F_4—A A A 35A: 1a 35A: 1a 3A: 1a a a

It is possible that a single set in an otherwise tetraploid plant may have an extra chromosome, giving 5 chromosomes in one set and 4 in the remaining eleven. At least we have a single plant in a tetraploid pedigree which strongly resembles the Globe—the best known of our simple trisomic mutants. The cytological evidence shows that its chromosomal number is at least tetraploid, but is not yet sufficient to prove that its Globe-like appearance is determined by the addition of a fifth member to the chromosomal set responsible for the Globe mutant.

The occurrence of mutations of the types discussed in the foregoing paragraphs is bound up with the causes of chromosomal duplication. Knowing the mechanism to be affected, we may be able ultimately to induce chromosomal mutations by the application of appropriate stimuli.

We have outlined the types of chromosomal duplication already found in the jimson weed, and have shown some of the peculiarities in the breeding behavior of the mutant forms which they condition. It will be well to consider for a moment this process of duplication as it affects the individual plant and as it may have a possible significance in our theories of mutation and evolution.

The mutants of the Poinsettia or Globe type, in which but a single chromosomal set is involved in the duplication, should enable one to discover something in regard

to the influence of each specific chromosome upon the morphology and physiology of the datura plant. While there seems to be but a single chromosomal set responsible for the presence or absence of purple pigmentation, probably each chromosome has an influence upon the strength of expression of the pigment since the several mutants appear to differ widely in color when homozygous for the main purple factor. Thus Glossy is darker purple than normals, while Cocklebur is distinctly lighter. In normal plants there is a balanced adjustment between the modifying factors in the different chromosomes. When this balance is disturbed by the addition of only a single extra chromosome to one of the 12 sets, profound changes are brought about in the ontogeny of the resultant plant. When all of the sets have an extra chromosome, however, as is the case in triploids, no great disturbance of the balance is brought about and the plant is not greatly different from normals. Even in tetraploid plants where all the sets are equally affected, although the total number of chromosomes is doubled, the difference from normal is not so great as in mutants of the Globe and Poinsettia series. The leaves of tetraploid plants, when carrying the factor for many nodes, may be distinctly larger than those of normals. Few-noded tetraploids, however, are less easily distinguished. The best diagnostic character has been the globose shape of the capsule, and yet plants known to be tetraploid from cytological evidence have been found this past season with capsules perfectly normal in appearance.

What is the bearing of the phenomena of chromosomal duplication in datura upon the mutation theory? In the first place, the mutants of the Globe type apparently correspond to the *lata* type of mutants in the Œnotheras in which an odd somatic chromosome has been determined, although in these Œnothera mutants no breeding evidence has been available to show that the peculiarities of mutant *lata* are due to the presence of an extra chromosome in any specific chromosomal set. Our tetraploid

mutant "New Species" corresponds to *Œnothera gigas* and is brought about by a doubling of the chromosome number. The color ratios in our tetraploid daturas indicate that *Œnothera nanella* is a Mendelian segregate and suggest that other of the Œnothera mutants which give monohybrid ratios in crosses may be of the same nature. Our evidence in regard to *O. nanella* comes from the occurrence of this mutant in cultures of *O. gigas*. DeVries (14) reports that certain races of *gigas* when selfed regularly produce from 1 to 2 per cent. *nanella* mutants, while certain pedigrees give monohybrid ratios which, on account of the lesser vitality of the recessive *nanellas*, show a higher proportion of the dominant *gigas* forms. From the pedigrees approaching a 3:1 ratio he obtained plants which bred true, except again for the 1 to 2 per cent. of *nanella* mutants in their offspring. A glance at the chart (Table III) will show that, if our theory of tetraploidy be correct, the 1 to 2 per cent. of mutant *nanellas* which deVries obtained by selfing plants from 3:1 pedigrees must have been the recessives in a 35:1 ratio since no dominant plants in a 3:1 pedigree of a tetraploid race could be expected to breed true. The dominant phenotypes must either throw 3:1 ratios again or 35:1 ratios. The deviations of the *nanella* mutants in this case from a 35:1 ratio is accounted for by a similar proportionate deviation in the 3:1 ratio. The work of Muller (8) on balanced lethals strongly suggests that such of the Œnothera mutants as are not caused by chromosomal duplication are due to cross-overs from a balanced lethal condition.

What then is a mutation? I do not feel we need to be bound by its application to the evening primrose for reasons of priority, since Waagen (15) had previously used the term in paleontology in an entirely different sense. I believe, with the idea that mutations must involve a qualitative change, that we shall ultimately confine the term to mutations of genes, although such mutations may later be shown to be as different from our

present conceptions of them as are mutations in the Œnotheras from the conceptions in deVries's classical publication, "The Mutation Theory." It may still be desirable to employ the word *mutation* as a collective term to designate the sudden appearance of any apparent genetic novelty—whatever its real cause—until we know better. Strictly speaking I should not call chromosomal aberrations mutations when the changes are purely quantitative. The occurrence of tetraploidy would therefore be no more a mutation than the doubling of chromosomes at the origin of the sporophyte from the gametophyte of ferns.

We have seen that chromosomal duplications and related phenomena may simulate gene mutations in their effects upon the individual. What is their possible significance in evolution? Let us first consider tetraploidy. Numerous investigators have called attention to the fact that the chromosome numbers of plants are more frequently in multiples of two and four than one would expect from random sampling. Pairs of related species have been listed for which one member had twice as many chromosomes as the other. Such species have even been called tetraploid. We feel strongly the desirability of confining the term tetraploidy to those cases in which the 4x number is brought about by a doubling of homologous chromosomes. Doubling by transverse division is a possible method, but would not be included in the term.

Tetraploidy has been observed in experimental cultures of œnothera, primula and datura. Do such tetraploid plants occur in nature, and are they capable of giving rise to taxonomically new species? It may be mentioned that the tetraploid datura was called "New Species" before its tetraploid nature was suspected. It satisfied the requirements of an independent species. The pollen was relatively good, and the mutant formed a distinct race, self-fertile and fertile *inter se*, while practically sterile with the parent stock. Tetraploid plants, therefore, stand slight chance of being swamped by hybridization with the

species from which they have sprung. Once arisen, their chances of survival would depend upon their ability to complete with other forms in the struggle for existence. There are no certain cases of tetraploidy known outside of cultivation. It must be admitted, however, that their identification would be difficult. I have shown that gigantism is not an invariable diagnostic feature of tetraploid daturas. As yet no cytological criteria of tetraploidy have been established. The breeding behavior, which is the only safe test, might easily be misinterpreted, as it was apparently by deVries in the case of the tetraploid *gigas* and *nanella*. Moreover, a suspected form must show a pair of Mendelizing characters before a breeding test can be applied.

Despite the paucity of evidence in regard to the occurrence of tetraploidy in nature, the speaker believes that it may have been one of the principal methods in the evolution of plants. Its occurrence would furnish the barrier between a new species and its parental form that Darwin sought, and it would give a reason for the prevalence of even numbers in the counts of chromosome pairs. I believe that a search for tetraploid forms in nature will be rewarded. Perhaps they will more likely be found in horticultural races propagated by vegetative means. I take this occasion to suggest the desirability of testing for tetraploidy any *gigas*-like plant that may be found in the wild or under cultivation. We are making a special study of tetraploidy at the Station for Experimental Evolution and should be glad to receive plants suspected of being tetraploid from any who do not care to make the necessary tests themselves.

Even if proper tests should show that few forms in nature were tetraploid in the sense that each chromosomal set in somatic cells was composed of 4 homologous members, tetraploidy might still be a stage in the origin of species with an even number of pairs of chromosomes. In the 3 forms in which tetraploid plants have arisen under observation, the 4 homologous chromosomes in a

set apparently assort at random in the reduction division. If, instead of acting individually without predilection one for another, the four should come to assort in pairs, we should have a different ratio in the F_2 generation (15:1 instead of 35:1). There would still be duplication of genes and a 4x number in reference to the parental form, but independent assortment of the chromosomes would have been lost. It will not be possible to go into the details of the argument. It is at least suggestive that Shull (12) has found 3 cases of duplicate genes in the shepherd's purse, which has 32 chromosomes (that is 4 times 8); and Nilson-Ehle (10) has found a case of triplicate genes in a wheat having 42 chromosomes, which is 3 times the number in another variety of wheat (11).

If tetraploid plants have been of influence in evolution, it is probable that the other types of duplication have also been of influence. A mutant of the Globe type with a single duplication in one of the 12 sets ordinarily fails to hand on the duplication through the pollen. Occasionally it might do so, and we should then expect a constant race with 4 homologous chromosomes in one of the 12 sets. If these 4 should cease to assort at random and pair, we should again have a possible duplication of genes and an added pair of chromosomes characteristic of the race.

There is not time at my disposal to discuss mutations of genes. In a recent paper (2) on a somatic mutation in portulaca, I have indicated my belief that mutations of genes may occur at any stage in the development of the plant. We have found color mutations which affected only the epidermis, and therefore could not be transmitted through seeds. We have also found similar color mutations which affected only the sub-epidermal tissue, and therefore could not show in the petal; but which became evident from the seeds produced from this mutated tissue. There seems to be no preferred location for the origin of factorial mutations in flowering plants, although they are more readily transmitted if they occur in the

gametes or in the embryo. The fact that in vegetatively propagated Mucors (3) I have found mutations relatively common where the possibility of sexual reproduction was ruled out, indicates that sudden genetic changes are not necessarily associated with sexual processes.

It has not been possible in this brief presentation to give an extended classification of mutations, nor to discuss in detail their possible significance in evolution. It will be sufficient if I have made clear the distinction which must be kept in mind, in any discussion of the subject, between mutations in individual genes and those brought about by chromosomal aberrations.

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BOOKS AND LITERATURE

Die Chromosomenzahl von Zea Mays L. Ein Beitrag zur Hypothese der Individualität der Chromosomen und zur Frage über die Herkunft von Zea Mays L.[1] By YOSHINARI KUWADA.

The author of the paper, the title of which appears above, has well summed up his purpose in the subheading. As this article reports investigations of considerable cytological importance in a publication which is not likely to have wide circulation in America, it was thought advisable to review it at some length.

As Professor Kuwada clearly and concisely states his results and conclusions in his summary a translation is given below.

1. The chromosome number of *Zea Mays* L. is 10 (when the diploid number is 20). In forms either closely related systematically or supposedly ancestral types the chromosome number in the root tips is in general constantly 20 (seldom does the number approach the octoploid number).

2. It has been found that in one race of sugar corn which I received from the Agricultural Faculty of the Imperial University of Tokyo the chromosome number is different in different individuals. In the roots tips 21, 21, 22, 23 and 24 chromosomes were found. The number of tetrads is correspondingly different, namely, 10, 11 and 12. There is no relation between the chromosome number and the chemical nature of the endosperm.

3. Through a parallel study of the number and size of the tetrads and the length of the chromosomes in the root tips it has been shown that the number of chromosomes is increased through the cross fragmentation of certain chromosomes.

4. The measurement of the chromosome length in the root tips and the unequal lengths of the component elements of the tetrads allow us to draw the important conclusion that *Zea Mays* is of hybrid nature, and indeed, as Collins has rightly said, a hybrid between Euchlæna and an unknown plant of the Andropogoneæ.

5. The chromosomes supposedly derived from *Euchlœna* are longer than those coming from the Andropogoneæ species, so that the tetrads under certain circumstances are made up of elements of different lengths. The two chromosomes of the first kind A—B[2] and C—D have

[1] *Jour. of the Col. of Science*, Imp. Univ. of Tokyo, Vol. 39, Art. 10, 1919.

[2] It has been necessary for convenience to take some liberties with the method used by Kuwada for expressing his idea of the potentiality of fragmentation possessed by the various chromosomes. Here a solid dash be-

an inclination to fragment easily under certain conditions while the corresponding chromosomes of the latter type a b and c d do not show this tendency. In one plant (sugar corn) from the agricultural faculty of the University of Tokyo the chromosomes A—B and C—D have each cross fragmented into two chromosomes A B and C D and this condition is morphologically and genetically fixed. We therefore have three kinds of corresponding chromosomes: the cross-fragmented chromosomes, those having a tendency to fragment and those in which both of these characteristics are lacking.

6. In the formation of the tetrads the chromosomes A B and C D are dominant to A—B and C—D and recessive to a b and c d. The dominance in the first case is somewhat unstable, so that the number of tetrads is subjected to fluctuation within certain limits. The difference in the behavior of the corresponding chromosomes A—B, C—D and a b, c d to A B C D is also a point in favor of Collins' hypothesis.

7. If the chromosomes A B and A—B form a tetrad in the reduction division four combinations result: A B, A—B, A—B and A– –B. The corresponding ends of the chromosomes A– and –B fuse relatively easily to reform the chromosome A—B. The possibility of fusion depends absolutely on the proximity of the corresponding ends of the passive cross-fragmented chromosome A– and –B. In this respect the parallel arrangement of the homologous chromosomes in the somatic cells is of great importance. The chromosomes A and –B or A– and B which would otherwise occasionally fuse to form the chromosome A and –B or A– and B remain sometimes as A and –B or A– and B: the result being the variation in the number of the chromosomes. Two kinds of gametes occur, in one the chromosome number is constant and in the other it varies. The chromosomes in the first instance have the formula A B (number of chromosomes above normal) or A—B (normal number of chromosomes), and in the latter instance A—B or A– B or occasionally A– –B. When the chromosomes A B and a b form a tetrad the result is very simple in that only two combinations are possible—A B and a b. In these cases the number of chromosomes is constant. The union of A and B is only a phenomenon ascribed to the presence of a b.

The empirical results agree with those developed from theoretical considerations based on the laws of chance.

8. The applicability of the laws of chance to the chromosome number between two letters indicates a weak place that may easily break, a broken dash before or after a letter suggests a free end of a fragment which will link up with the suitable end of another fragment if opportunity offers, underscored single letters have no power of uniting (no free ends), while the binding together of two letters by underscoring represents a chromosome which can never fragment.

ber and the constancy of the true length of the chromosomes in the hybrids is a contribution favoring the individuality hypothesis.

9. The nuclear and cell size is dependent on the chromosome size and on the other hand the latter is modified by the cell size.

According to Kuwada there are two hypotheses concerning the origin of *Zea Mays* L. Iltis (1911) first suggested that this modern form might have been derived from some unknown tribe of Andropogoneæ, while Collins in 1912 put forward the claim that *Zea Mays* L. was a hybrid between an unknown species of the Andropogoneæ and *Euchlæna*.

In his cytological studies Kuwada finds that in species of *Euchlæna* and *Andropogoneæ* the chromosome number is the same as in *Zea Mays* L.—namely 20. In only one of the investigated groups of plants belonging to the *Andropogoneæ* was the chromosome number above 20, which places this particular species beyond consideration. The measurement of the chromosomes in a *Euchlæna* from south Florida shows that their total length is greater than is the case in *Andropogon Nardus* L. var. *Georingin* Hack. The respective total chromosome lengths in each case are given as 188.25 mm. and 111.3 mm.

Kuwada gives the results of a large number of measurements of the chromosomes in various varieties of maize taken at random or from plants in which the cytological conditions have been studied in the parental, F_1 and F_2 generations. His conclusion that the figures indicate that two length types of chromosomes are concerned in the modern plant do not seem to the present writer to be entirely born out by the facts.

In the measurement of chromosomes previous studies have shown that complexes from the same individual in the same or in different parts of the structure may show considerable variation in the total length of their component chromosomes. In general, of course, small cells will have smaller chromosomes and larger cells, larger chromosomes, but even in similar tissues very appreciable differences may occur. These variations are obviously due both to internal and to external causes. Fluctuation in the climatic or nutritive conditions may affect growth and vigor, while the volume of the cell imposes limitations on the size of the contained chromosomes. It has been shown by the present writer[3] that, be the total length of a complex long or short, the

[3] Hance, R. T., 1917, "The Diploid Chromosome Complexes of the Pig (*Sus scrofa*) and their Variations, *Jour. Morph.*, Vol. 30. 1918*a*, "Variations in the Number of Somatic Chromosomes in *Œnothera scintillans* De-

individual pairs always bear the same relation to each other, allowing the conclusion that whatever influences the size of the chromosomes generally affects all similarly. The figures of Kuwada bear out these observations very well. As pointed out above, his interpretation of his work seems somewhat forced. He recognizes the factors playing rôles in the behavior of the chromosomes, but does not feel that his results can be entirely explained by them.

To illustrate what is meant by the above criticism let us consider a cross made by Kuwada between sugar corn $22_{(15)}$ and Black Mexican $58_{(15)}$, another sugar corn. In both, dividing cells from adventitious roots were uniformly selected. The former has chromosome complexes averaging 149.05 mm. in length while the latter gives a total of 172.17 mm. This to Kuwada indicates a real and genetic difference in chromosome length, although in the same Black Mexican plant $58_{(15)}$ complexes from side root tips average only 145 mm. in length. This would signify that the length 172.11 was no more fundamental in plant $58_{(15)}$ than was 145, and lessens the weight of the evidence that the higher number betokens genetic chromosome differences with the length 149.05 in plant $22_{(15)}$. When the two plants are crossed the chromosome lengths in the hybrids are almost exactly one half of the sum of the lengths of these structures in the parents if 172.17 is accepted as the typical complex length for plant $58_{(15)}$— $1/2(149.05 + 172.17) = 160.61$. It may be pointed out here that one half the sum of the complex length found in the various roots of plant $58_{(15)}$ also closely approximates the same figure— $1/2(172.17 + 145) = 158.58$. The F_1 plants from the above cross possess sets of chromosomes whose length is very close to that expected on Kuwada's assumption of 149.05 and 172.17 as the basic or typical lengths of the parental chromosomes. The chromosomes in the F_1 plants varied from 155.75 mm. to 168.9 mm. and averaged 161.86 mm. This number fits in well with the anticipated result and at first would seem to justify the consideration of 172.17 mm. as the representative length for plant $58_{(15)}$. However, the chromosomes in the F_1 offspring were found in cells in the radicles of seed germinated in moist saw dust. The chromosomes in this early root tip in many forms are not infrequently larger than are found in the growing parts of

Vries, *Genetics*, Vol. 3. 1918*b*, "Variations in Somatic Chromosomes," *Biol. Bull.*, Vol. 35.

the older plant and Kuwada's figures and statements show that maize is no exception to the general rule. This tendency for the chromosomes in the radicle to be larger puts a fictitious value on their measurements in this organ for comparison with the dimensions of chromosomes found elsewhere in the plant. As a matter of fact, in the number of examples given the average length of the chromosomes in all the plants is only a trifle more than one per cent. shorter than the similar data in regard to the chromosomes of the radicle, which difference would not greatly affect the end result. In this instance, although the physiological location of the chromosomes was undoubtedly one factor in determining their size, objection on this ground alone to the submission of the records of the F_1 chromosome lengths in substantiating the figure 172.17 as the fundamental chromosome length for plant $58_{(15)}$ would not seem to be entirely valid. However, to base a broad conclusion on the lengths of the chromosomes found in a particular part of a plant, even though comparing them with chromosomes from similar parts of other plants, is likely to obscure the real condition.

As has been shown in plant $58_{(15)}$, lengths of 172.17 mm. and 145 mm. were found. That these are not fixed lengths for the particular tissues concerned in this variety of corn is shown by the data given for other plants of the variety Black Mexican, in which lengths vary (for corresponding tissues) from 132.5 mm. to 181.25 mm., the average being 159.32 mm. There can be little question that the variety Black Mexican, as long as it is genetically pure, can have anything but comparable sets of chromosome throughout, holding in mind that though the lengths may vary the inter-pair relationship remains constant. Less variation in chromosome length is shown for the three plants of the variety "Sugar Corn" which were studied. The range of averages here is from 147.8 mm. to 151.6 mm.

Lastly, if real differences between the lengths of the chromosomes in plants $58_{(15)}$ and $22_{(15)}$ exist greater differences between the members of the pairs that are found in the hybrid offspring would be expected. Actually these elements mate up well as to length and if unequal homologous chromosomes have entered the zygotes union in a common environment has regulated their proportions. As the dimensions of the chromosomes are in part a function of their environment the selection as typical of any one complex or of even the average of com-

plexes from certain tissues only is not justified, considering our present ignorance of chromosome volume.

In support of the difference in length between the homologues of chromosome pairs as indicative of the genetic length types which Kuwada believes he has demonstrated he publishes drawings of tetrads showing the unequal length of the component elements. Personally I do not think that the figures are necessarily conclusive proof, since the arrangement of the homologues in several cases suggests a possible foreshortening, making the true length doubtful, and in other instances the drawings may well represent an entirely different form or stage of the tetrad. It is not the intention of this criticism to convey the impression that the investigator's figures fail absolutely in proving his point concerning the uneven length of the homologues, but rather to indicate that the illustrations are not nearly as satisfactory and as conclusive as those given in the publications of Wenrich[4] and Carothers[5] for somewhat similar conditions in other forms.

Between the *Euchlæna* and Andropogoneæ studied chromosome length differences appear which can scarcely be accounted for on the basis of environment. Since the characteristics of *Zea Mays* L. are intermediate between these forms the hope is raised that two sets of chromosomes will be found in the modern species, which hope the reviewer does not think has been realized. Indeed, though recognizing the evolutionary position of *Zea Mays* L. as given by some taxonomists, he offers the suggestion that in his opinion the present investigation has not, as far as the chromosomes are concerned, excluded the possibility of the origin by mutation of *Zea Mays* L. from *either Euchlæna* or the Andropogoneæ. A knowledge of the behavior of the chromosomes of these two forms in hybrids would be interesting and important.

In explanation of the variation in the number of chromosomes which Kuwada found in certain lines (20 to 24 chromosomes) he devised an exceedingly ingenious scheme which apparently thoroughly accounts for the numbers of chromosomes occurring in the offspring. It operates on the laws of chance and its theory

[4] Wenrich, D. H., 1916, "The Spermatogenesis of *Phrynotettix magnus* and the Individuality of the Chromosomes, *Bull. Mus. Comp. Zool.*, Harvard College, Vol. 60.

[5] Carothers, E. E., 1913, "The Mendelian Ratio in Relation to Certain Orthopteran Chromosomes," *Jour. Morph.*, Vol. 24.

seems to be completely justified by the results. As this explanation is adequately outlined in the translated summary further space need not be devoted to it.

The investigator's theory of factors located in each chromomere which govern the form of the chromosome, while convenient in explaining the cause of the reunion of the chromosome fragments in maize, is scarcely necessary. Chromosomes are not inherited as are the determiners for adult characteristics in the form of minute chemical forerunners, but are passed on complete in all respects. Consequently, factors to determine their form in the next generation are not needed—the chromosome itself is carried over. The actual form of the chromosome has been shown by McClung, Wenrich, Carothers and others to be determined largely by the location of the spindle fiber attachment.

It is considered that the reviewed report has not clearly demonstrated the origin of *Zea Mays* L. by means of chromosome measurements for the following reasons:

1. The length of the selected chromosome complexes in the forms particularly studied are not typical of the plant and such selection gives a false impression of the actual conditions.

2. The figures illustrating the length differences of the homologues composing the tetrads are not entirely convincing or satisfactory.

3. If two types of genetically fixed chromosome lengths exist in maize we would expect to find an expression of this difference when both types enter into the same individual. As far as the reviewer's interpretation of the tables of length is concerned, this difference does not exist in the F_1 plants.

Though there are reasons for not considering that Kuwada has proved his claims of the origin of *Zea Mays* L. he, nevertheless, is to be sincerely congratulated on an excellent cytological contribution involving great labor and care. To the reviewer the apparent failure of Professor Kuwada to demonstrate his main thesis dwindles in importance when the value of the "side issues" of the investigation are considered. His work on *Zea Mays* L. presents the following data:

1. The chromosome pairs of a complex may be arranged in a graduated length series and between each pair there is an approximately equal difference in length.

2. The genetic relation of the chromosomes is shown in parent and offspring.

3. When chromosomes fragment in *Zea Mays* L. it is the longer ones that are affected. These fragments may also fuse, causing variability in the total chromosome number.

4. Suggestive methods of studying chromosomes have been devised.

5. Fragmentation has been accounted for on the basis of genetic tendencies and the variable number of the chromosomes in the offspring of certain plants has been ingeniously explained with the aid of the device described in his summary.

The first four points (with the exception of the latter part of the third, which has not been observed) agree perfectly with the reviewer's earlier work on the Œnotheras and the pig. As to the fifth point, he has never found fragmentation in the germ line.

Difficulties of interpretation in metrical studies of chromosomes arise from a lack of standards, *i.e.*, knowledge of the limits of variation that chromosomes of a given form will show under many conditions and of the uncertainty introduced by the personal equation involved in drawing and measuring. With the hope of deriving such standards the present writer is at work on a plant and an animal possessing very few chromosomes. The usefulness of the information drawn from such studies has been elsewhere discussed.

ROBERT T. HANCE

ZOOLOGICAL LABORATORIES,
UNIVERSITY OF PENNSYLVANIA.

SHORTER ARTICLES AND DISCUSSION

"HOMING" BEHAVIOR IN CHITON[1]

1. A STATEMENT concluding a recent preliminary account of the "homing" habits of the pulmonate *Onchidium floridanum* (Arey and Crozier, 1918) reads as follows:

> To the extent that the homing habits of *Onchidium* may be proved to involve associative memory, this snail may be placed in a series comprising such types as *Chiton*, *Fissurella*, *Onchidium* and *Octopus*, all four of which, in a sense, exhibit homing behavior, but of increasing degrees of precision and complexity in the order of arrangement here given.

The observations warranting a contention of this sort, so far as it involves *Chiton*, were not fully available when our analysis of the sensory responses of *Chiton tuberculatus* (Arey and Crozier, 1919) was written, and I have therefore considered it appropriate, as an addition to that report, to indicate the nature of the facts leading us to ascribe to *Chiton* a certain degree of "homing" behavior.

2. It was noted by Heath (1899, p. 4), on the Californian coast, that the adult *Ischnochiton magdalenensis* is found during the day under boulders between tides, but that at night the mollusc comes out to feed on seaweeds growing upon the rocks, retiring to dark situations after sunrise. Species of *Mopalia*, and *Cryptochiton*, were found to "remain out on their feeding grounds only when the day is foggy or dark." Numerous other species are more or less photonegative (cf. also Crozier, 1919*b*), but some nevertheless continuously occupy situations brilliantly illuminated (cf. Heath, 1899, p. 4; Plate, 1901; Pelseneer, 1906, p. 50). It has been shown elsewhere (Crozier and Arey, 1918; Arey and Crozier, 1919) that young individuals of *Chiton tuberculatus* are photonegative to ordinary daylight, the older ones photopositive. This matter of photic irritability is intimately concerned with certain diurnal movements simulating "homing" behavior.

Heath (1904) was of the opinion that the bilateral larval eyes of some chitons, persisting as they do well into postlarval life, until the shell plates become opaque, might be functionally im-

[1] Contributions from the Bermuda Biological Station for Research. No. 127.

portant in determining responses to light. Earlier, he had noted (1899, p. 4) that the ventral soft parts of *Ischnochiton*, especially the proboscis, might be sensitive to light, and it has been stated in a general way (Pelseneer, 1906, p. 50) that chitons in which there are no obvious shell "eyes" seem, nevertheless, to be sensitive to illumination. Direct proof has, however, been given by Arey and myself (1919) that the tegmental æsthetes of *Chiton tuberculatus* are photosensitive, and that this form of irritability is important in determining the habitat of an individual chiton.

C. tuberculatus attains a mean age of about 8 years (Crozier, 1918*a*, 1918*b*). As it grows, the periostracum and the surface of

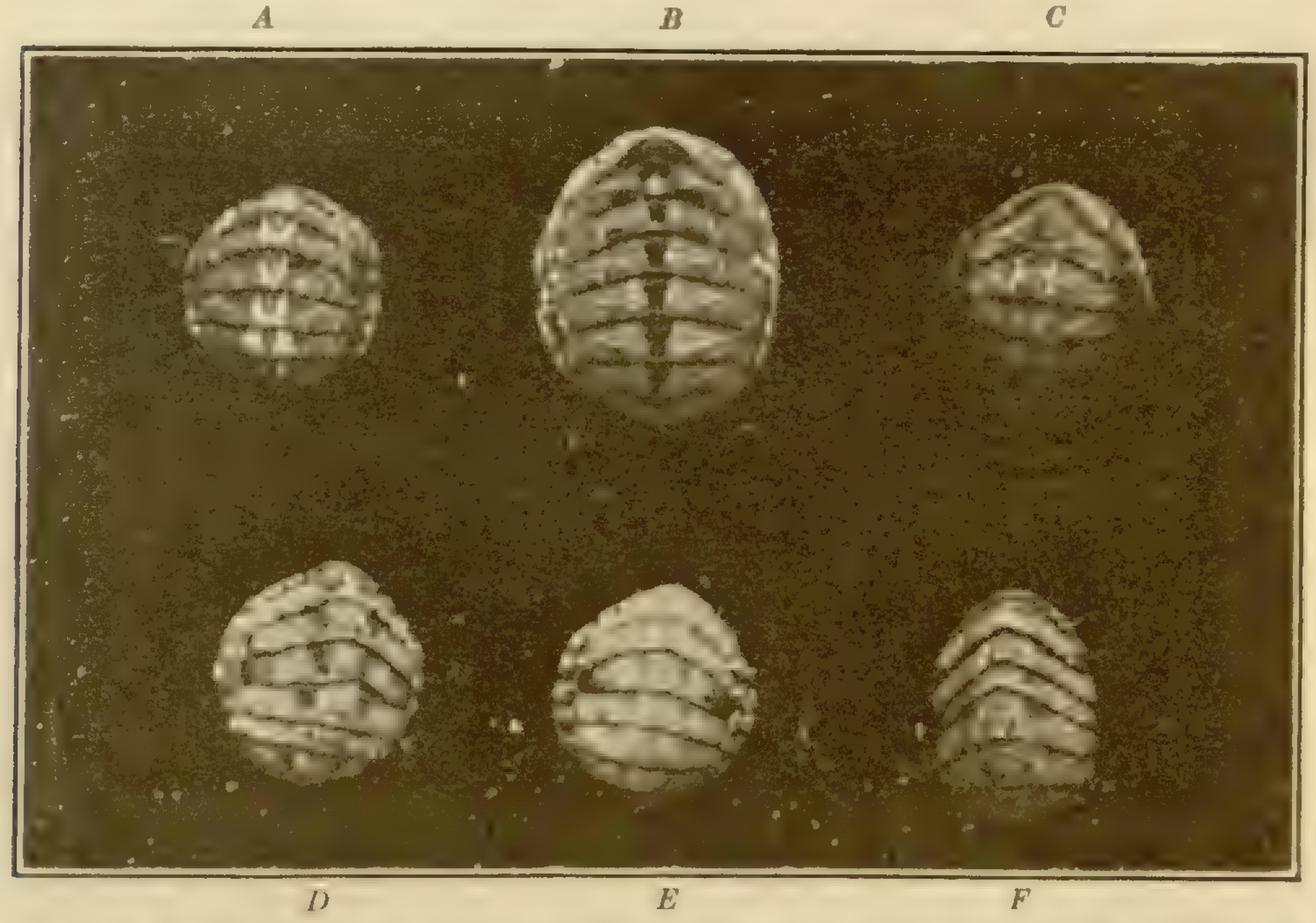

FIG. 1. Illustrating the relative lengths, ages and degrees of shell-erosion in three groups of *Chiton tuberculatus* taken from localities quite near together, but in the different situations indicated. The extent of erosion seen in each specimen was estimated by comparison with a graded series of "standard" chitons: *A*, signifying *no erosion; B, slight erosion; C, a more severe stage, but mild in comparison with D and E,* the last representing *relatively extreme destruction of the tegmental surfaces.*

the tegmentum become eroded, and the superficial photoreceptive organs destroyed (Arey and Crozier, 1919). There is an almost perfect correlation between the degree of this erosion and the relative illumination of the situations frequented by these chitons. The analysis of this state of affairs, and its implications, will be more fully considered in another place, but as an illustration I cite the following record, which is quite typical of many others:

July 1, 1918. At the northwest end of Marshall, Idaho, a small cove

bounded on the west side by rocks exposed to the sunlight of a cloudless day; the cove covered by loose slabs of rock, piled upon one another; both situations, the exposed rock surface and the under sides of the loose stones, yielded a number of chitons.

From the sunny rock surface, 38 individuals were obtained, ranging in length from 5.5 to 8.9 cms., and in estimated age (Crozier, 1918*a*) from 5 to 11 years. These were without exception seen to have the tegmenta eroded to a greater or less extent.

From several crevices in the rock, having their deeper recesses well sheltered from the sun, 8 specimens were secured. These were 4.5–6.9 cms. long, 4–6 years old, and but slightly eroded.

Under the stones, 30 chitons were collected. There were 3.4–6.9 cms. long, 2–7 years old, and at the most very mildly eroded.

Fig. 1 exhibits, for these lots, the distribution of: (1) length, (2) age, and (3) degree of erosion. The "degree of erosion" of the shell plates was judged empirically by comparison with a graded series of "standard" examples, a method sufficiently precise for the purposes of this illustration.

It is obvious from the figure that those chitons with relatively uneroded shells are younger, smaller, and live in darkened situations; whereas the older individuals, larger, with much eroded shells, occur in the bright sunlight; those taken in partially illuminated cracks and crevices are of intermediate size and age, and their shells exhibit an intermediate degree of erosion,[2] these characteristics affording, in fact, an ethological definition of a certain portion of the chiton population. The individuals of this general "intermediate" class are frequently so situated that they exhibit the type of "nocturnal" activity noted by Heath (1899) for several species—they creep over open rock surfaces, feeding, at night, and may remain out there on dark and gloomy days, but return to crevices (or to the partial shelter of large stones) when the rising sun is of ordinary brilliancy.

The uneroded chitons are photonegative to moonlight, even, and although moving about actively at night, do not provide data bearing on the possibility of "homing." This is largely true because the photonegative response of the younger, uneroded, chitons becomes altered, depending upon the destruction of the tegmental receptors, in the directions of a photopositive reaction to ordinary daylight; this alteration depends, not on age, but upon

[2] There are several methods of estimating rather precisely the exact amount of erosion in any given case. In a later paper these methods are fully made use of in analyzing the observed distributional occurrence of the Chitons.

the degree of intactiveness of the photoreceptive apparatus, and its importance in the present connection is due to the fact that the chitons of intermediate age, eroded to a moderate degree, and less photonegative[3] than the smaller ones, come to occur in places where moderate illumination prevails. Moreover, they move about much more freely than the younger individuals, thus often getting some distance away from loose rocks, not plentiful along many stretches of shore line. Crevices of one sort or another, or shaded depressions, are therefore the one type of refuge open to them.

3. It was found, by observing a group of marked chitons each day for a month, that the older ones and those of the "intermediate" group do not wander readily from place to place (Arey and Crozier, 1919). Another group containing 14 chitons of the "intermediate" class as above defined was under daily observation for three months, and the behavior of this group strongly suggested a feeble kind of "homing" phenomenon. The animals concerned spent most of the day under a boulder at half-tide. At night they crept out for distances of not more than a meter, feeding on *Enteromorpha* and other algæ. With the rising sun, they retreated to the rock-shelter. If the tide were out at sunrise, they remained more or less fixed until again covered by the sea, then moving toward the rock.

This sort of behavior, regularly and constantly exhibited, seemed to represent perhaps the incipient stages of a kind of "homing instinct."[4] The movements of the individuals of this group were therefore carefully watched. The bit of shore con-

[3] As worked out in a previous report (Arey and Crozier, 1919), the partial destruction of the photoreceptive apparatus through erosion is a principal factor conditioning lower stimulating power of light of a given intensity; in general, *Chiton* is actively photopositive to weak light, negative to intense light; with advancing age, therefore, the threshold for photonegativity becomes higher.

[4] Data regarding the movements of a single *Chiton* favorably situated on the side of a wharf and watched continuously for 9 months are given elsewhere (Arey and Crozier, 1919). This animal was quite old, and very much eroded. It remained in the open except during severe storms and one hurricane, when waves beat fiercely on the wharf. Under these circumstances the mollusc withdrew to a cavity at one end of the wharf, near the shore-line, and remained hidden. Several other cavities were available, but this particular one was automatically encountered as the *Chiton* moved shoreward on the wharf-side and away from the more exposed outer edge of the wharf. One wonders what the anectodotalist would make of a case like this!

cerned faced in general southeastward, and the tiny platform over which the chitons crept while feeding was so oriented with reference to the rising sun that the photonegative orientation of the animals and their subsequent creeping brought them for the most part automatically back to the shaded hollow under the rock. But I noticed repeatedly that in some instances the molluscs moved at an angle of 30°–40° across the direction of the sun's rays, moving more or less directly toward the rock. If such individuals were suddenly detached (with the aid of a cold-chisel and hammer, removing the animal still fixed to a bit of stone), and so placed as to necessitate its approaching the rock at a different angle, it usually did so without trouble. If removed to a greater distance than 1.5 meters, no return was effected, the creature taking up a more or less permanent site in another shaded hollow.

Aside from light, it must be remembered that there are other possible directive agencies in such a case. The sea was rarely still, and even a slight tidal current would be sufficient to reflect pressure waves from the shore,—so that, perhaps by this means in part, a chiton would be oriented toward shore, and thus, in the present case, inevitably toward its rock-pocket. Additional specimens of the general "intermediate" group, brought from distant islands, were "planted" in this community, and engaged in the same nocturnal wanderings and early morning returns.

4. Without further analysis, the activities of a group of Chitons such as that described, may seem to involve a sort of behavior resembling the well-known "homing" of *Fissurella*[5] and its allies. Yet with *Chiton* the matter is clearly less definite than in other instances recognized among molluses, and, so far as I have seen, the facts may readily be interpreted in terms of immediate directive stimulation. There is nothing necessarily *specific* about the Chiton's "home." For this very reason such homing movements as *Chiton* may exhibit at a certain period of

[5] I encountered a curious instance of the "local habitation" affected by limpets, when examining the chitons of a reef on the south shore of Bermuda. One of these chitons bore on its back a small *Fissurella*, the margin of the shell of which had become so modified that it fitted nicely one particular spot on the much curved surface of the third valve of the chiton. Under water, the *Fissurella* wandered over the eroded shell of the large chiton, feeding upon epiphytic growths, but always returned to its "home." The *Chiton* was 9.3 cms., the *Fissurella* 0.9 cm. long.

its life-history may be taken to represent one extreme in the development of such behavior among molluscs, seen perhaps in its highest condition in *Octopus* (cf., *e.g.*, Cowdry, 1911).

W. J. Crozier

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AN F_1 SPECIES CROSS BETWEEN HORDEUM VULGARE AND HORDEUM MURANIUM

Within the last few years the subject of species hybridization has increasingly occupied the attention of those interested in the subject of heredity. The possibility of the genetic analysis of species hybrids depends upon the ability to cross and to secure offspring from the species in question. During the course of a plant-breeding investigation which was commenced at the

University of California, an attempt was made to determine if the common cultivated barley could be crossed with wild species of *Hordeum*. The wild species which were used were *H. nodosum* and *H. muranium*. For the sake of convenience the system of nomenclature proposed by H. V. Harlan (1918) will be adopted for the common varieties of barley used in the investigation.

One of the crosses which was attempted was between *H. vulgare vulgare pallidum* and *H. nodosum*. This cross was an entire failure, however, as no seeds were obtained from any of the flowers which were crossed. The anthers of the male parent were fully mature, and the plant which was used for the female parent was perfectly healthy and normal when the cross was attempted. As a matter of fact a successful cross was made the same day between a different head of the same plant of *H. vulgare vulgare pallidum* and *H. vulgare distichon palmella*. At the present time it would be difficult to say whether the absolute failure of this particular cross was due to the incompatibility of the gametes of the two parents or to certain errors of technique.

The other cross which was attempted was between *H. vulgare trifurcatum typica* and *H. muranium*. The contrast between the two parents was very marked and distinct. The low and often recumbent habit of growth of *muranium* was contrasted with the relatively tall and erect habit of *vulgare*. The light green leaves and stems of *muranium* were not nearly as stout as the gray green leaves and stems of *vulgare*. The spikes of the two species are also quite distinct. The spikes of *muranium* are compressed and composed of a number of rather narrow elongated spikelets which form rather a loose head. The spikes of *vulgare*, on the other hand, are generally composed of a number of relatively short and wide spikelets. Both species are annuals, but without going into further detail it is evident that there are a large number of morphological differences between these two species.

From the second cross two viable hybrid seeds were obtained. These grains resembled the typical seeds of the maternal variety in every respect. When they were planted, however, it required one and three days longer for the seed to germinate than for self-fertilized seed of the female parent.

The F_1 seedlings differed markedly from plants of *H. vulgare* in the same stage of development. The sheath or coleoptile had a greater circumference than the blade, thus fitting loosely

around it instead of adhering closely to the blade as in *vulgare*. The sheath was closed along the side and open only at the apex. The blade of the first leaf was narrow, linear and spirally twisted with slightly roughened edges. The blade was about one twelfth of an inch in width and tapered slightly toward the apex (Fig. 1).

One plant grew to a height of four inches and developed roots two to three inches long (see Fig. 2). The other plant developed somewhat more slowly, reaching a height of two inches

FIG. 1. A first generation hybrid between *Hordeum vulgare trifurcatum typica* and *Hordeum muranium*.

with roots of the same length. At this stage the plants ceased development and gradually started to wither. Only one blade was present and this extended to the seed. There was no evidence of any nodes being formed.

Due to a change of residence the writer has not been able to continue the investigation for a short time, but it is hoped that this cross may be subjected to further breeding tests as well as a histological examination. The theoretical hypotheses concerning species crosses have been thoroughly reviewed by other writers (Babcock and Clausen 1918), but it may not be out of place to briefly state the particular theories which probably account for the results considered in this paper.

It has already been pointed out that *H. vulgare* and *H. muranium* differ in a large number of morphological characters.

H. muranium may be considered as a monotypic species, and the slight variations which are found in the species are undoubtedly due to the effects of the environment and would be classed as non-heritable variations. *H. vulgare*, on the other hand, is a polytypic species consisting of many varieties which differ in a number of morphological characters. Most of the factors which condition the characters of *vulgare* display har-

FIG. 2. F_1 species hybrids between *Hordeum vulgare* and *Hordeum muranium* at the stage of growth at which development ceased.

monious interrelations with one another and mendelize in a normal fashion. Several factors involving chlorophyll reduction have been discovered, however, and these genes have been found to be incompatible with the normal functioning of the chromatin system. In these cases after the food material in the seed has been exhausted the seedlings usually die, for the change

in the factors has been too far reaching to give a normal functioning reaction system.

This brings forth the theory of reaction systems which has been thoroughly reviewed by Goodspeed and Clausen (1917a). The purpose of the discussion in the preceding paragraph was to show that both *vulgare* and *muranium* possess a normal reaction system, and second that a normal reaction system may sometimes be disturbed by lethal factors. When we attempt to combine two distinct reaction systems, however (and the distinct morphological characters of the two species as well as the breeding test would indicate that the two species possessed different reaction systems) an inharmonious group is often formed which fails to function in a normal fashion. In the case of chlorophyll reduction there is one or at most only a few factors disturbing the reaction system. In the case of species crosses there are a number of factors, which in all probability differ qualitatively, coming from two distinct reaction systems and these often fail to harmonize. The results are often similar, however, for the differences between the reaction systems of *vulgare* and *muranium* are so profound that the resulting system is not able to function after the food material in the seed is exhausted.

The type of species cross described in this paper is quite similar to the species cross between *Crepis capillaris* and *Crepis tectorum* recently described by Babcock and Collins, 1920. The two species of *Crepis*, besides differing in several morphological characters, were found to differ in chromosome number. Reciprocal crosses gave equivalent results, or the dominance of *tectorum* cotyledon characters in F_1 accompanied by hybrid vigor. The F_1 seedlings died, however, in every case at the end of the cotyledon stage. Cytological examination revealed a complete lack of order in the cell systems, and as a result these systems failed to function and development ceased. The species cross in barley involves slightly greater contrasts perhaps than those in *Crepis* but both give nearly parallel results.

There are, as has been pointed out by others, all degrees of incompatibility of reaction systems in species crosses. The range of compatibility includes cases of complete or nearly complete fertility, as in the species crosses in *Antirrhinum* (Baur and Lotsy), examples like those found in *Nicotiana* (Goodspeed and Clausen 1917*b*) where the fact of incompatibility does not become evident until the fertilization of the F_1 plants, and

finally we have species which exhibit complete incompatibility by refusing to cross with one another. The range includes many intermediate conditions like those found in *Crepis*, which nearly approach complete incompatibility. The cross between *H. vulgare* and *H. muranium*, then, is well down the scale and can be grouped in the class with the two species of *Crepis* as showing nearly complete incompatibility.

WALTER SCOTT MALLOCH

AGRICULTURAL COLLEGE OF TEXAS

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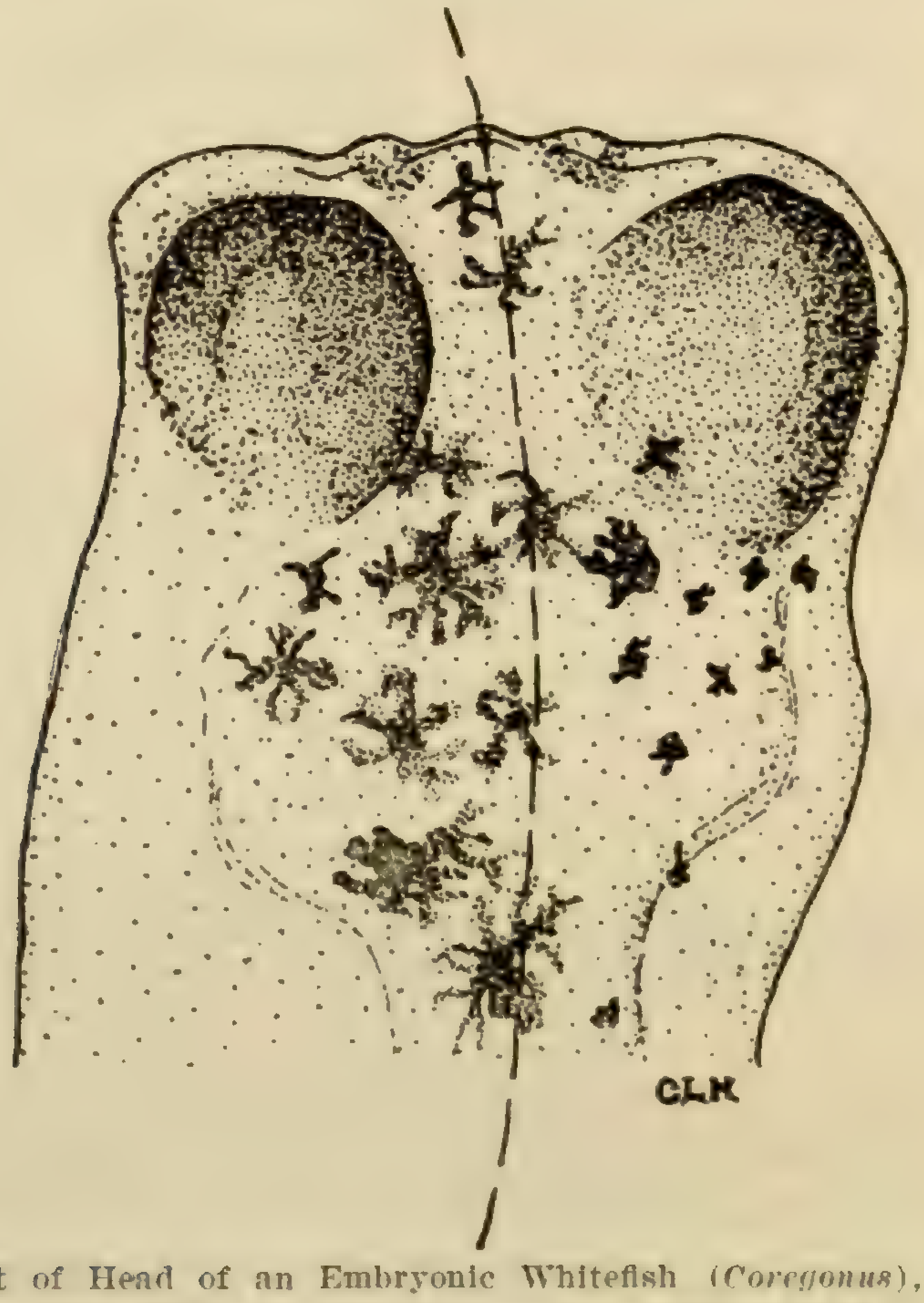
A NOTE ON UNILATERAL REACTIONS OF THE MELANOPHORES OF THE HEAD IN FISHES

IN most discussions of the physiology of the chromatophores of fishes it is apparently assumed that the reactions are strictly bilateral, *i.e.*, synchronous on the two sides. The writer, however, has lately observed a number of cases in which the reaction was either unilateral or imperfectly bilateral.

Upon death, the melanophores of one side of the head in some cases become all "contracted" to the extreme, while those of the other side become widely "expanded." As a result, one side of the head becomes very pale, the other side blackish, the two areas being abruptly opposed along the mediodorsal line. This notable

color change at death has been observed by the writer in an adult pike (*Esox lucius*); in a young-of-the-year of the shiner (*Notropis cornutus*); and in embryonic and larval whitefish (*Coregonus clupeaformis*) and lake-herring (*Leucichthys ontariensis*).



Dorsal Aspect of Head of an Embryonic Whitefish (*Coregonus*), to Illustrate the Unilateral Reaction of the Melanophores.

This phenomenon is not confined to death, however, as the following observations demonstrate. A nuptial male of *Pimephales notatus* (a minnow in which the head becomes densely charged with black pigment during the breeding and nesting activities), apparently normal in respect to its eyes and other structures and functions, found guarding its eggs, had one side of the head abruptly pale. Similarly embryonic and larval coregonine fishes were repeatedly observed to have the melanophores expanded only on one side of the head during life. In the case of the male *Pimephales*, no change in the pigmentation of the head was noted while the fish was being observed for several minutes, nor upon its capture, death or preservation.

In other cases the unilateral reaction of the melanophores was less permanent, appearing as a transient phenomenon; due perhaps, to a differential reaction rate of the chromatophores of the two sides. Two experiments[1] illustrative of this point may be cited.

1. A live, normal, apparently healthy embryo of the lake whitefish (*Coregonus clupeaformis*), developed approximately to the hatching stage, was found to have the dorsal melanophores considerably "expanded" on the head, slightly expanded on the body. Following the removal of the egg envelope, under approximately unchanged conditions, these color-cells "contracted" in this order: (1) body, (2) left side of head, (3) right side of head. Still under similar conditions, the cephalic melanophores again expanded, those of the left side most widely. No further change could then be induced, even by rather intense light-heat stimulation, until the left eye was dissected out and the embryo again held before the light. Reaction occurred at once only on the left (now the blinded) side, the lateral melanophores contracting more rapidly and more completely than the inner ones; as in the first instance, reaction followed (some time after the removal of the stimulus) on the right side, the melanophores contracting in the same order as on the left side.

2. A similar embryo of the same species had the dorsal melanophores of the head well expanded when removed from its egg envelope. The pigment granules of all melanophores on the right side then rapidly migrated into the center of the cells, under observation. No reaction occurred on the left side, even following stimulation with a bright light, although this caused first a partial contraction and then a re-expansion of the right chromatophores. Reaction on both sides was finally accomplished by sudden transfer of the eggs from water near room-temperature (about 25°) to water at 1.7° C., but even in this case the contraction was more complete and rapid on the right than on the left side.

CARL L. HUBBS
UNIVERSITY OF MICHIGAN

[1] These experiments were incidental to other studies which the writer carried on during the winter of 1919–1920 in the bionomics laboratory of the University of Chicago; he desires to thank Doctors Lillie and Bellamy of that institution for the opportunity they kindly afforded him to do this work.

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FURTHER DATA ON THE INHERITANCE OF BLUE IN POULTRY[1]

PROFESSOR WILLIAM A. LIPPINCOTT

KANSAS AGRICULTURAL EXPERIMENT STATION, MANHATTAN, KANSAS

I. PREVIOUS WORK

The principal facts concerning the genetic behavior of blue in the Andalusian breed of domestic fowl were presented in an earlier paper (Lippincott, 1918*a*). Previous work on the genetics of the blue Andalusian was reviewed and a limited number of further data were offered.

The latter showed that blue Andalusians are like black Andalusians in that they are self-colored. They are, on the other hand, like the blue-splashed Andalusians in that homologous pigmented feathers in both sexes have the same condition with reference to the restriction of pigment in the feather structure. The 1:2:1 ratio obtained from mating blue Andalusians together may be interpreted as the combination of two 3 to 1 ratios. These relationships are shown in Fig. 1.

The restriction of black pigment in the feather structure to give the blue appearance found in blue and in blue-splashed Andalusians was shown to be due to the action of a dominant factor *R*. The extension of black pigment to all feathers of the body as in both black and blue Andalusians, was found to be due to the action of another dominant factor *E*.

[1] Contribution from the Department of Genetics, Wisconsin Agricultural Experiment Station, No. 29, and from the Department of Poultry Husbandry, Kansas Agricultural Experiment Station, No. 15.

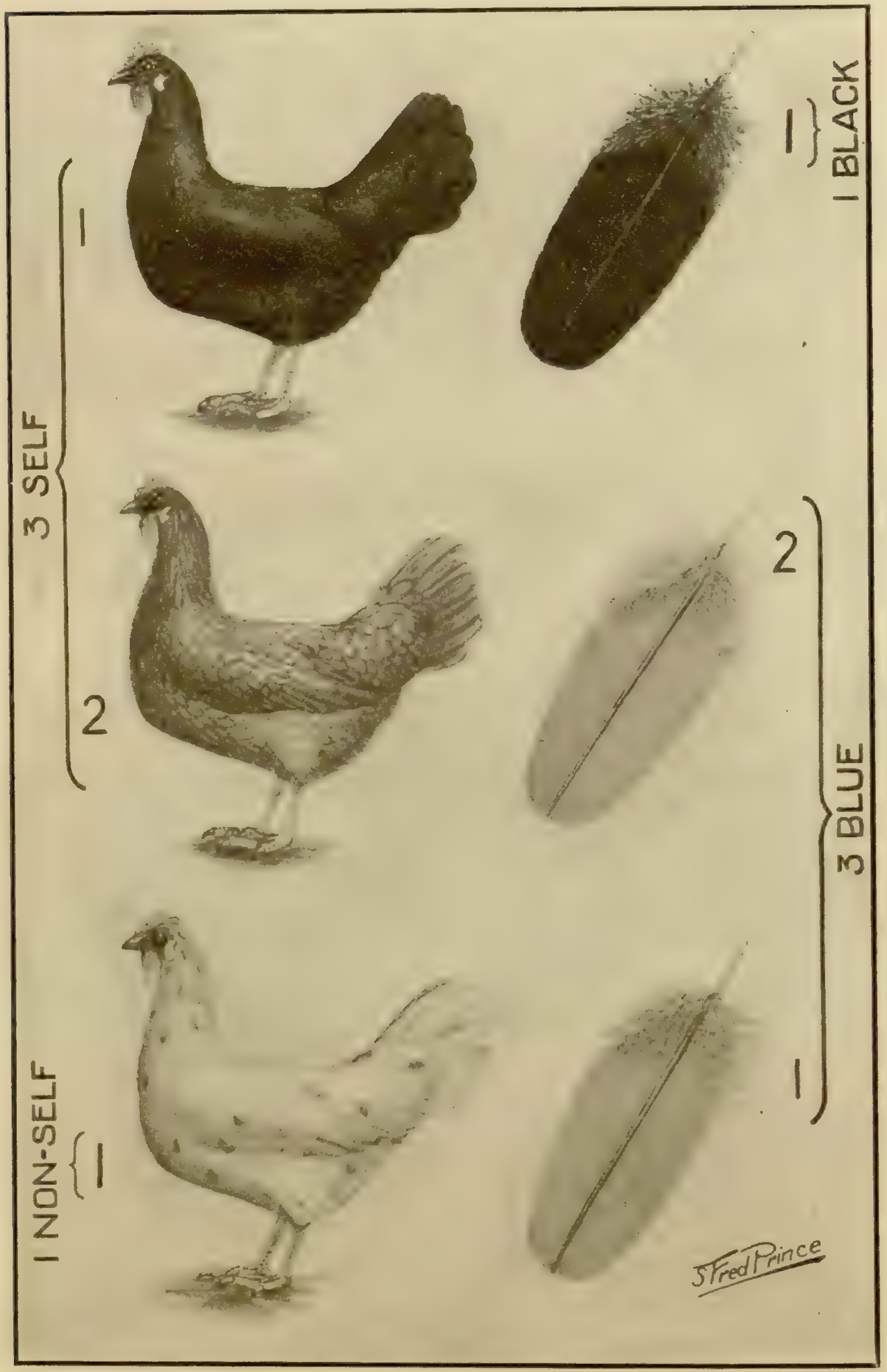


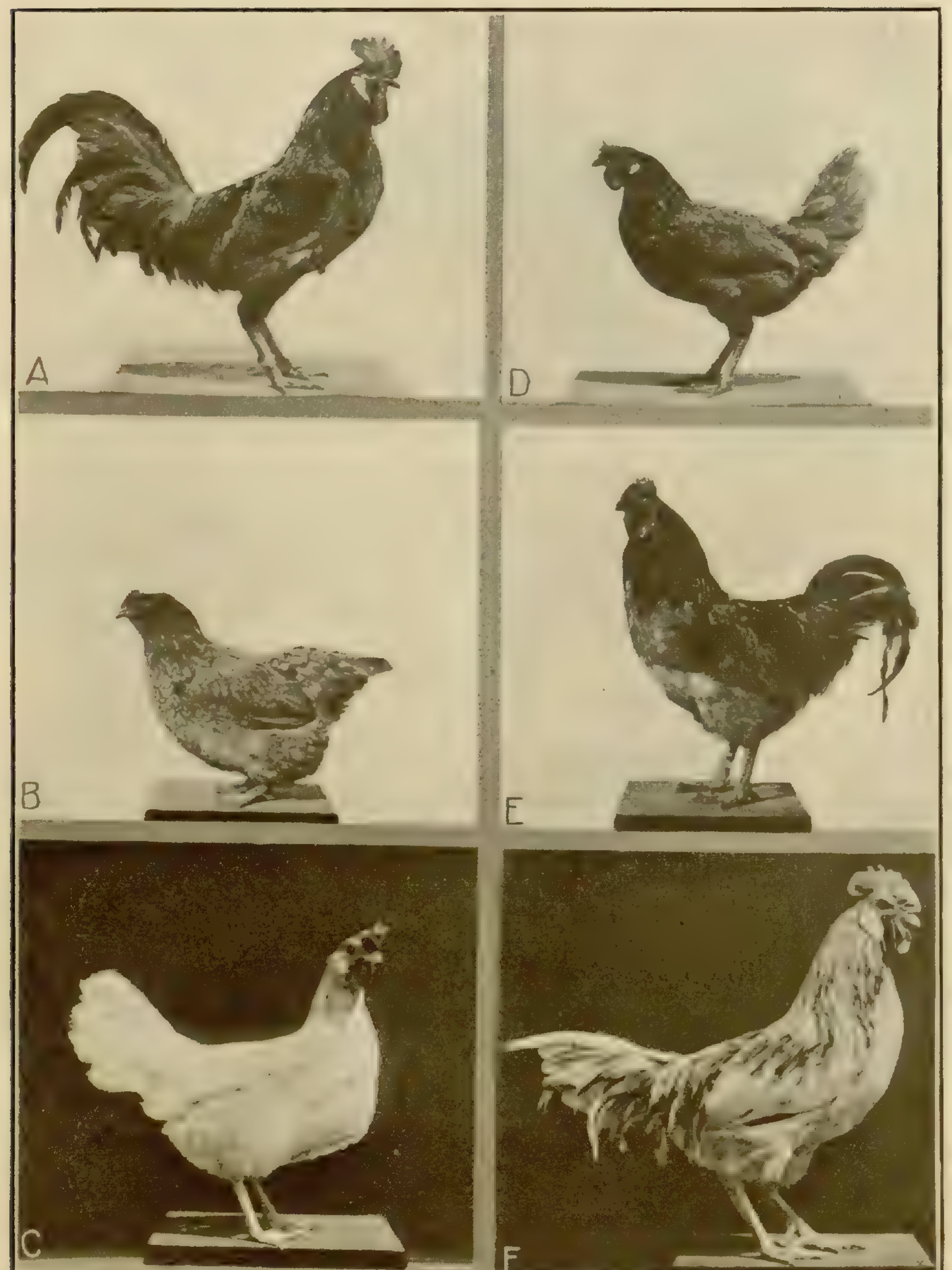
FIG. 1. Showing that the 1 : 2 : 1 ratio is a combination of two 3 : 1 ratios.

It was pointed out that while, on the basis of their expression in the phenotype it appeared more logical to consider these factors as dominants, each closely linked to the recessive allelomorph of the other, they may, so far as the experimental evidence shows, be considered as true allelomorphs occupying identical loci on homologous chromosomes, and each expressing itself independently of the other.

The finding of crossovers between *R* and *E* would be conclusive evidence proving the former of the two conditions proposed. It was shown that while no crossovers had been reported the critical data on the case were very limited and the likelihood of crossovers being detected and isolated by breeders is very small. It might well have been suggested further that even though crossing-over does rarely occur, for instance, so that less than one per cent. of the individuals are the product of crossover gametes, the chances of detecting them experimentally are small, considering the limited number of matings (as determined by the equipment available at most experimental institutions) which are likely to be devoted to a search for crossovers.

Though much has been made of the blue Andalusians as a "heterozygote phenotypically intermediate between the parental types" it was shown that while all self-blues so far found had proved to be heterozygous for *R* and *E*, they were not in the strict sense intermediate between the parental types. The F_1 progeny of a cross between blue-splashed Andalusians and white Wyandottes was reported as self-blue and far darker than either parent.

It was further shown in the earlier paper that *R* not only restricts black pigment, so as to render pigmented areas bluish-gray in appearance, but also affects the shape of the pigment granules, so that instead of appearing as rods as in black individuals, they are quite round. In this particular *R* is quite dominant over its allelomorph, whether one chooses to assume that the latter is *E* or *r*.



EXPLANATION OF PLATE I

The photographs shown in Plates I and II were taken by James Machir, my indebtedness to whom it is a pleasure to acknowledge.

FIG. A. Blue Andalusian male.
FIG. B. Blue Orpington female.
FIG. C. Blue-splashed Andalusian female.
FIG. D. Blue Andalusian female.
FIG. E. Blue Orpington male.
FIG. F. Blue-splashed Andalusian male.

A and *D*—Blue Andalusian.
B and *E*—Blue Orpington.
C and *F*—Splashed Andalusian.

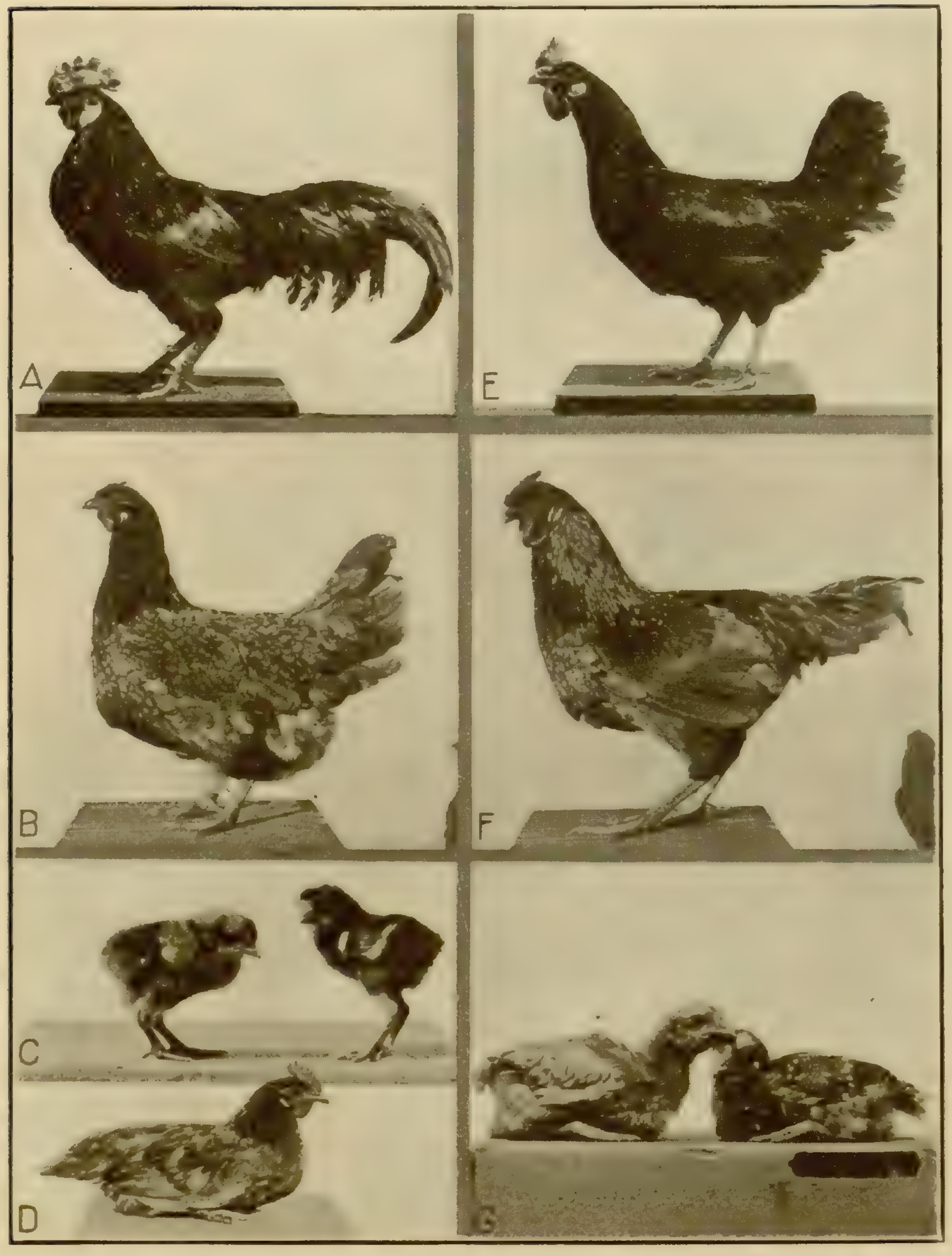
It was also shown that both the restricting and the rounding actions of *R* were interfered with in certain regions of both blue-splashed and blue males. In both color types the pigmented feathers of the neck (hackle), back, and saddle are black or bluish-black instead of blue as on the remainder of the body. The black pigment granules in these regions are for the most part rod-shaped rather than round. It was suggested that this interference with the action of *R* is a secondary sexual characteristic, presumably due to the presence of testicular or the absence of ovarian influence.

II. Purpose of the Present Paper

It is the purpose of this paper to present further data concerning the inheritance of blue and its relations to the sex glands, and to draw such conclusions as these data justify. A report is given of the breeding behavior of blue as found in the Andalusian, Orpington and Leghorn breeds, and of certain crosses of these breeds with each other and with other breeds, which do not possess blue varieties. The relations of the factors involved to certain factors present in the non-blue varieties of other breeds is considered and evidence concerning the relation of the sex-glands to the action of the factor *R* presented.

III. Material and Methods

The breeding stock used was from several sources, being in part from the pedigreed flock of the University of Wisconsin, where the work reported in the earlier paper was done. It was also in part from the pedigreed flock of Kansas State Agricultural College where the investigation was continued under the direction of Dr. Leon J. Cole of the University of Wisconsin, my indebtedness to whom it is a pleasure to acknowledge. The stock was, however, mostly from unpedigreed lines, though pure-bred within the meaning of the poultryman. In no case were individuals used which were not from families show-



EXPLANATION OF PLATE II

FIG. *A*. A black Andalusian male.

FIG. *B*. A blue F_1 female from a white Wyandotte × blue-splashed Andalusian cross.

FIG. *C*. A blue (at left) and a black (at right) chick in the down. These are offspring of a white Plymouth Rock ♂ × blue Andalusian ♀. The occipital spots inherited from the sire are plainly visible.

FIG. *D*. A young blue F_1 male from a blue-splashed × black Langshan cross.

FIG. *E*. A black Andalusian female.

FIG. *F*. A blue F_1 male from a white Wyandotte × blue-splashed Andalusian

ing the characteristics of their respective varieties with constancy in so far as could be learned. In as much as only varietal (color), as opposed to breed (shape) characteristics were being studied, less attention was paid to the latter in selecting material. In no case, however, were individuals used which showed disqualifying breed characteristics.

With a single exception no individual was used whose genotype proved to be inconsistent with the "breeding true" of the variety to which it belonged, or, in the case of the blue-splashed Andalusian, the variety from which it arose. This single exception was a blue-splashed Andalusian female (2107) purchased from a breeder who made only blue × blue matings. She proved to be heterozygous for *P*, a factor necessary for the production of black pigment. The family from which she arose must have been producing occasional whites which were, in all likelihood, being discarded as extremely light blue-splashed wasters from the blue × blue matings. This point was not followed up, however, and the facts ascertained. It has been by taking advantage of situations similar to this one that white varieties have been established in several breeds.

There were several individuals discovered whose factorial composition varied from the normal, or usual, for the varieties to which they respectively belonged. Owing to the particular factorial complex of which they were a part, however, these factors behaved as cryptomeres, not affecting the adult phenotype of the variety. Specific reference is made to these individuals in a later section of this paper.

The matings were, for the most part, made in covered

cross. Indications of a factor or factors for lacing may be seen in the hackle and saddle feathers.

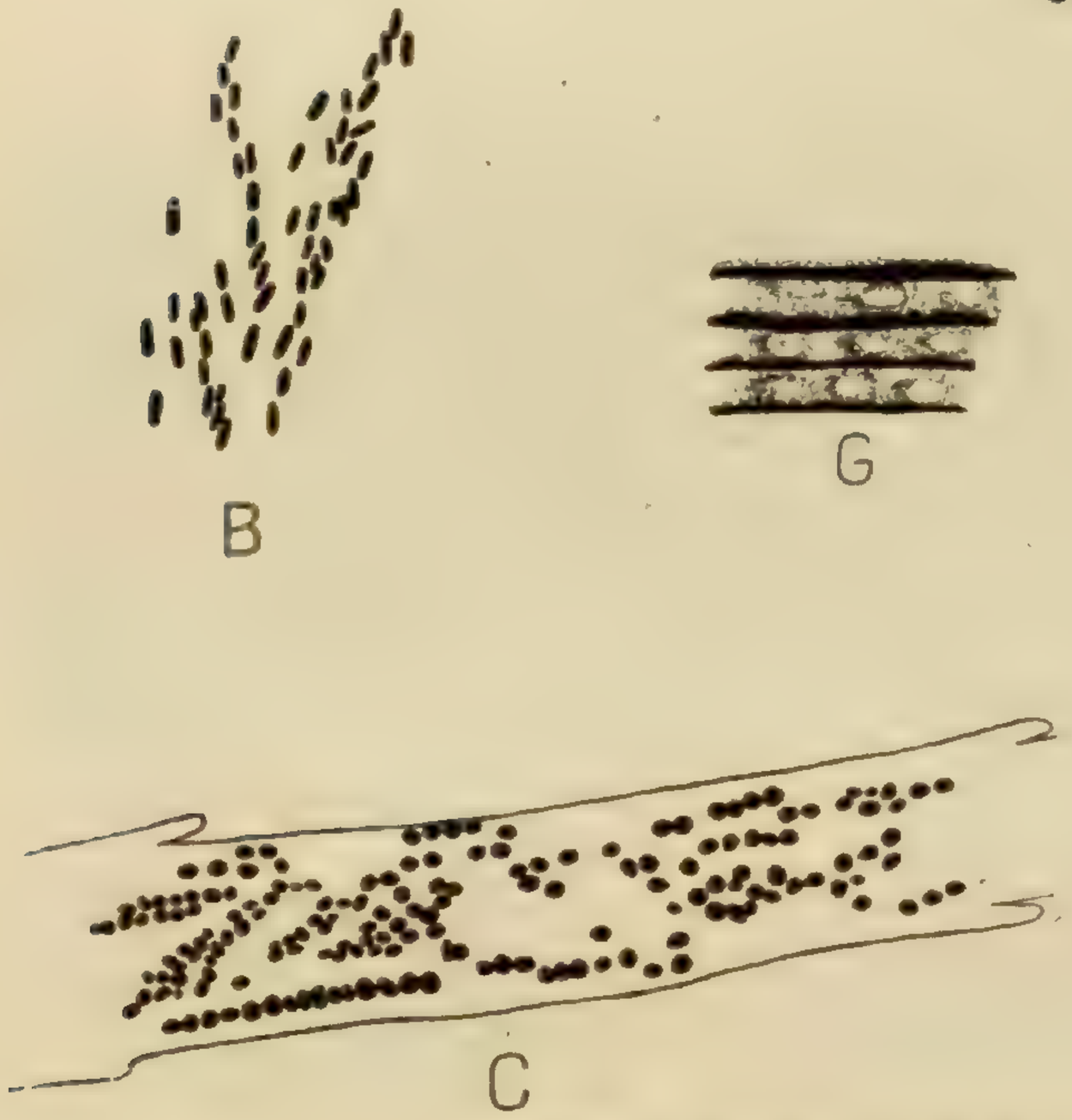
FIG. *G*. A blue-barred (at left) and a black-barred (at right) chick partly feathered. These are offspring of the same mating as the chicks shown in Fig. *C* this plate. The barring was inherited from their white Plymouth Rock sire.

A and *E*—Black Andalusian.

B and *F*—Blue F_1's.

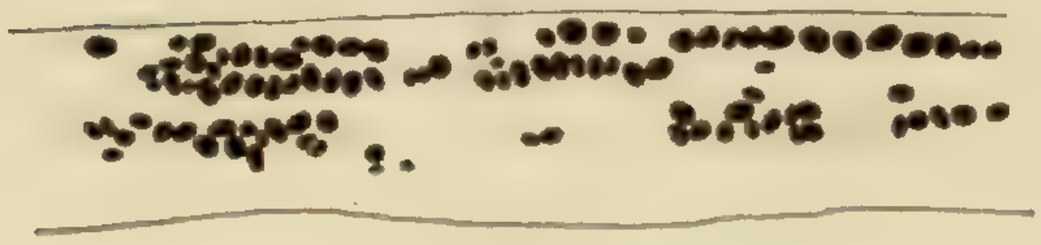
A

B

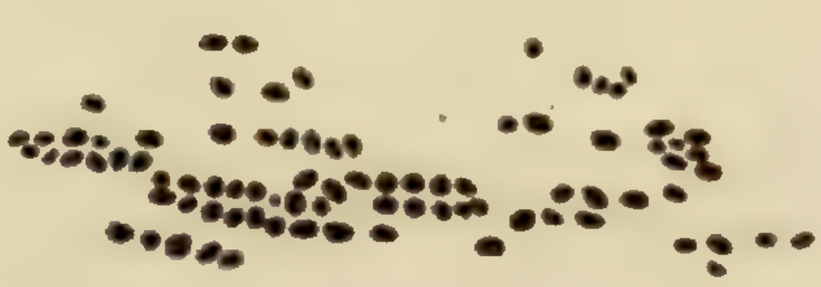


C

D

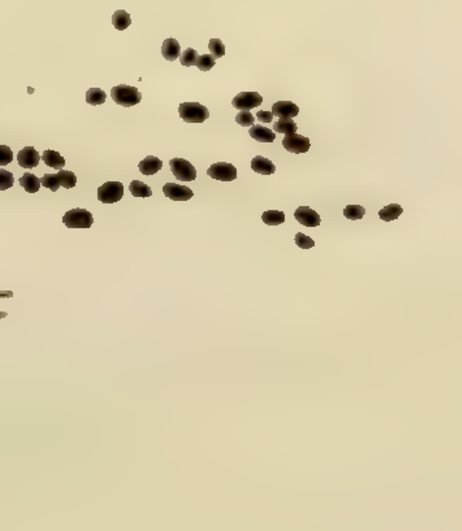


E



F

G



H

EXPLANATION OF PLATE III

FIG. A. Appearance of pigment granules in a strand of down from a very dark blue crossbred chick. The sire was a blue F_1 from a white Wyandotte × blue-splashed Andalusian cross. The mother was a blue Andalusian. There are occasional rod-shaped granules. Camera lucida drawing.

FIG. *B*. Appearance of pigment granules in a definitive feather from a black Andalusian. Granules of the same shape are found in the down and definitive feathers of black Langshans, black Orpingtons and the black offspring from crosses with the several breeds used in this investigation. Camera lucida drawing.

FIG. *C*. Appearance of pigment granules in a strand of down from a blue

yards and every precaution taken to insure no mixing of matings and the proper identification of the eggs laid by each female in each mating. Not only was the assistant in charge of the trapnesting selected because of his habitual accuracy in details, but the eggs from each individual hen were kept together, separate from the eggs of other individuals, and carefully compared one with another before being put into the incubator. Any off type or off colored eggs were discarded, so far as these experiments are concerned. In spite of these precautions it is too much to hope that some errors have not crept in, though it is believed they are very few.

Owing to the fact that the original stock was of relatively unknown composition, it was necessary to make such matings as would not only throw light on the behavior of the factors under observation, but would also be likely to bring to light unsuspected factors whose action might interefere with the action of the genes being studied. This necessitated introducing test females in the matings where the males were uncertain, and of mating many of the females with test males a second season instead of repeating the mating already made. In both cases the result was to reduce greatly the numbers of offspring from some of the crucial types of matings, and considerable numbers of "test" offspring were hatched and described, for the reporting of which here there is no particular object.

The counts of living chicks were made in the down at hatching time and individual descriptions recorded, each

F_1 chick from a white Wyandotte × blue-splashed Andalusian cross. Camera lucida drawing.

FIG. *D*. Clumped appearance of pigment granules in a curved barbule from a definitive feather of a blue Andalusian.

FIG. *E*. Appearance of pigment granules in a strand of down from a blue F_1 chick from a blue-splashed Andalusian × white Plymouth Rock cross. Camera lucida drawing.

FIG. *F*. Appearance of pigment granules in a definitive feather from a blue Andalusian. Camera lucida drawing.

FIG. *G*. A small area of the web of a definitive feather from a black Andalusian. The cell boundaries and nuclei may be made out. There is no clumping of pigment within the cells.

FIG. *H*. Appearance of pigment granules in a definitive feather from a blue Andalusian. Camera lucida drawing.

chick being marked with a numbered wingband. The system of keeping pedigree records in use has been described elsewhere (Lippincott, 1918*b*) and need not be repeated. The descriptions were checked when the chicks were three weeks old, and again at some considerably later, though not specified, time, when the birds were leg-banded for the breeding-pen or laying-house, or were sent to market. The descriptions of all chicks dying were carefully checked at the time they were found, though a small number disappeared without their descriptions being checked. Unless there was reason to suspect that their classification might be likely to change after the taking of the first description such individuals were counted.

Fortunately the different classes of offspring could for the most part be distinguished in the down, and counts were accordingly made of chicks which reached an advanced stage of development but which failed to hatch. It was the practise to test all eggs for live germs at the end of the tenth day of incubation and remove all infertile and dead eggs. A second testing was made on the eighteenth day when all the dead eggs found were opened, the embryos described and their sex recorded. On the twenty-second day, after the hatch was well over, all the eggs which failed to hatch were opened and the descriptions of the dead chicks made a matter of record.

In most cases the embryos from crosses among the three color types of Andalusians which passed the first test, developed far enough so that the differentiation between color types could be made with precision after the down had been carefully washed, and dried with the aid of an electric fan. In those crosses involving recessive white parents, only those unhatched chicks could be counted which lived past the eighteenth day.

There were two possible sources of confusion in the classification of the chicks in the down. These were the differentiations between blacks and occasional very dark blues, and between blue-splashed and recessive whites.

The blacks and dark blues could quite readily be separated by examining the down of each chick microscopically. The blacks carry only rod-shaped pigment granules while in dark blue down rounded granules predominate. These are frequently arranged in rows as reported in my former paper (1918*a*, pp. 98, 99). In the case of every mating where this method of classification was brought into use to aid in distinguishing individuals which failed to hatch, down samples were saved at hatching time from the living dark blue and black chicks as well, and the first description, the record of the microscopic examination, and the later descriptions after definitive feathers were developed were carefully compared and checked. In nearly all cases the descriptions in the down and in the definitive feathers agreed.

In certain matings, however, it was found that descriptions in the down were not reliable and could not be counted. This was particularly true of one family of Andalusians which carried considerable red in the plumage and which has been referred to by Platt (1916) and Pearl (1917) in other connections. Within this family and its crosses the expression of the *R* factor in the heterozygote was frequently delayed so that individuals which were described in the down as blacks and which showed only rods under the microscope turned out to be blues when the definitive feathers appeared, and then showed the characteristic round pigment granules of the blue. None of the chicks tracing their ancestry to this family are included in the counts herein reported.

The possible source of confusion in the classification in the down of the blue-splashed and the white chicks arises from the fact that while the adults of the white Plymouth Rocks and white Wyandottes are pure white, or very slightly flecked with black, the chicks frequently carry considerable, though varying amounts of black pigment in the down, which gives certain regions a bluish appearance. This varies in degree from near black to slightly

smoky white. Fortunately for the problem in hand the localization of this pigment in the down of certain regions of the body is quite characteristic and quickly recognized. While a blue-splashed chick is frequently very light blue, as noted by Bateson and Punnett (1906, p. 20), the pigment is not localized on the top and back of the head, the wings in the region of the bow, and on the thighs, as it is on the potentially white chick, and the impression conveyed is very different. In potentially white chicks the remiges, which may be seen just starting to grow out from their follicles, are pinkish white and exhibit not the slightest trace of pigment. In the same feathers of the blue-splashed chick, on the other hand, there is a very noticeable bluish cast and usually at least one remex that is distinctly pigmented.

Though in pure-bred white Plymouth Rock and white Wyandotte chicks the pigment granules in the down are typically rod-shaped this fact is not of assistance in classifying with respect to white and blue-splashed offspring from crosses involving the factor *R*, since under its influence black pigment granules are round whether in a potentially white or a blue-splashed chick.

Not all chicks from pure-bred white Plymouth Rock and white Wyandotte matings exhibit this juvenile pigment. Some can only be recorded as white. It is of interest that the only chicks, three in number, which were originally described as "white, no pigment" or "creamy white" and later used in a breeding pen, have all proved to carry a factor for dominant white, as described in a later section of this paper. The number of such birds which have been tested is small and no general conclusions can be drawn, but the results are suggestive. It is rather interesting to note that a photograph of a group of white Plymouth Rock chicks in "The Plymouth Rock Standard and Breed Book" (American Poultry Association, 1919, p. 419), which is the official guide for the breeding and judging of all Plymouth Rocks, shows individuals

which are noticeably pigmented. In response to a letter of inquiry Professor Arthur Smith of the University of Minnesota, the editor of this book, tells me that my observation concerning the presence of pigment in these chicks is correct and he adds in substance that the pigmented chicks develop into the whitest adults.

The fertility and hatching power of the eggs from the various crosses here reported and the viability of the chicks hatched was increasingly disappointing from season to season. While the comparative coefficients of fertility and hatching power have not been calculated, the ratio between the eggs set and chicks hatched has undoubtedly been lower on the average, than for the pure-bred unrelated matings of the same and other breeds, set in the same incubator at the same time, and certainly lower than would be counted satisfactory in ordinary poultry husbandry practise.

The foregoing applies as well to the rate of mortality. As representative of the numbers surviving to grow definitive feathers in comparison with the counts recorded in the various tables, those of the F_2 from the blue-splashed Andalusian ♂ × white Wyandotte ♀ may be given. The counts made when the chicks were feathered were 47 blue, 18 blue-splashed, 37 black, and 42 white. The total count recorded (see Table IV, group 1) was 100 blue, 46 blue-splashed, 65 black, and 64 white. The reasons for the low hatchability and high mortality have not been established.

Until considerably more data than are now available have been secured it seems best to call attention to the possibility of crossing-over between the loci of *R* and *E* by indicating their possible recessive allelomorphs. It is accordingly the practise in this paper to indicate these factors thus: (*Re*) and (*rE*).

IV. The Relation of Phenotype to Sex

It is convenient to consider the relation of phenotype to sex before examining the progenies of the various

matings. In order to secure evidence concerning this relation, six blue Andalusian males were caponized during the summer of 1919. Into the body cavities of three of them ovarian tissue from nearly related females was introduced, the other three being kept as checks. The operating was done on July 24 and the birds turned out on range with hundreds of other birds one week later. On September 19 one of them (wingband 1387) was killed by a skunk. At that time it was entirely blue, there being less contrast between the regions that are dark in the male (hackle, back and saddle) and the other regions of the body than frequently appears in blue pullets before comb development indicates the approach of the first laying cycle, and indeed in many mature females. Although it was over four months old (hatching date, May 6, 1919) it appeared so much like an immature pullet that it was mistaken for one by the poultryman in charge and by the writer, until its record and description were consulted. Concerning the latter there did not seem to be any chance of error, since the scar made in opening the body cavity was plainly visible.

Such a situation indicates a fairly complete molt between July 24 and September 19. This is not surprising, however, since Rice, Nixon and Rogers (1908, p. 66) have shown that "from the incubator to the laying period the chicks experienced at least four molts, either partial or complete," and it is further well known that a close relation exists between molting and ovarian activity.

The other birds operated on at the same time were at once looked up and described. One of them (wingband 1855) was found to be somewhat intermediate in condition, some of the feathers of the neck and saddle being blue, but somewhat darker in shade than the normally (in the male) blue regions of the body. There were, however, a few scattered feathers which were almost black from the tip halfway down the web toward the fluff. About midway between the tip of the feather and the be-

ginning of the fluff there was a distinct line of demarcation where the black or near-black became a distinct blue. This chick was hatched a little over two weeks later (May 22) than 1387 and had apparently not gone through a complete molt, some feathers in process of growth at the time of the operation and showing ovarian influence on the last regions to develop still remaining.

On October 26 this bird was killed, apparently by a rat, at which time all of the feathers of the neck and saddle regions were distinctly blue, though considerably darker than other parts of the body. The shape of the feathers was characteristically female.

The third male into which ovarian tissue was introduced (wingband 1480) showed no influence of the introduced tissue on September 19. This condition still prevailed when it was sent to market October 26. It appeared normal for a blue capon of that age, over five months, the hackle and saddle being very dark and characteristically male in shape. Presumably the ovarian tissue introduced atrophied without having any effect.

Of the cockerels which were caponized, but had no ovarian tissue introduced, one (wingband 1859) died soon after the operation. The other two (wingbands 1415 and 1492) showed and continued to show typical blue capon characteristics with regard to the color and shape of the saddle and hackle feathers. The feathers were fully as dark as in normal males of the same age, and as they matured were even longer than their homologs in normal males. This result is precisely the same as that observed by the writer several times in blue capons, concerning which no descriptive records were kept.

In this connection it should be observed that in the family of Andalusians here dealt with, it has been not infrequently noticed that certain nearly grown pullets whose combs have not begun to develop, show only very dark feathers in the regions of the neck and back. These same birds after their combs begin to redden, thereby

indicating ovarian activity and the onset of laying, appear to pass through a molt or partial molt whereby the dark feathers of the back region particularly, are gradually replaced by those of a clearer blue. The necks of such females usually remain dark, showing considerable contrast with the other regions of the body, though being by no means as dark as the same region of the blue male.

Although the number of desexed males into which ovaries were introduced was small, it seems fair to conclude in the light of the evidence concerning testicular (Goodale, 1916) and ovarian (Goodale, 1918; Cole and Lippincott, 1919) influence in fowls that the failure of the factor *R* to express itself as fully in the neck, back and saddle regions of the blue and blue-splashed males as in the females is due to the lack of some necessary cooperative action on the part of the ovary, and not to any inhibitive action on the part of the testis.

V. The Breeding Behavior of Andalusians

New data concerning the breeding behavior of the three color types of Andalusians, as shown by several types of matings, are presented in Table I.

TABLE I

SHOWING THE NUMBERS AND COLOR TYPES OF PROGENIES FROM VARIOUS ANDALUSIAN CROSSES[2]

Group	♂ ♂	♀ ♀		Blue-spl. (*Re*) (*Re*)	Blue (*Re*) (*rE*)	Black (*rE*) (*rE*)
1..	Blue (*Re*) (*rE*)	× blue (*Re*) (*rE*)	Obtained...	46	104	64
			Theoretical.	53.5	107	53.5
2..	Blue (*Re*) (*rE*)	× black (*rE*) (*rE*)	Obtained...	00	25	24
			Theoretical.	00	24.5	24.5
3..	Black (*rE*) (*rE*)	× blue (*Re*) (*rE*)	Obtained...	00	113	90
			Theoretical.	00	101.5	101.5
4..	Blue (*Re*) (*rE*)	× blue-splashed (*Re*) (*Re*)	Obtained...	1	1	0
			Theoretical.	1	1	0
5..	Blue-splashed (*Re*) (*Re*)	× blue (*Re*) (*rE*)	Obtained...	35	33	0
			Theoretical.	34	34	0
6..	Black (*rE*) (*rE*)	× blue-splashed (*Re*) (*Re*)	Obtained...	0	138	0
			Theoretical.	0	138	0
7..	Blue-splashed (*Re*) (*Re*)	× black (*rE*) (*rE*)	Obtained...	0	56	0
			Theoretical.	0	56	0
8..	Blue-splashed (*Re*) (*Re*)	× blue-splashed (*Re*) (*Re*)	Obtained...	0	12	0
			Theoretical.	0	12	0

[2] Andalusians are normally homozygous for *P*, a factor necessary for the production of black pigment.

These results are in substantial accord with those of Bateson and Punnett (1906, p. 20). A somewhat marked departure from the theoretical expectation appears in group (3) of black ♂♂ × blue ♀♀ matings, the agreement in the reciprocal cross (group 2) being as close as possible. This departure from expectation is due to the progeny of a single pair of birds (♂136M and ♀2005) which produced 25 blues and 6 blacks. If the latter are left out of consideration the results are 88 blues and 84 blacks.

However, even in the case of the progeny of ♂136M and ♀2005 the Dev./P.E. = 4.1, which indicates a deviation of doubtful significance. The results of this mating were carefully considered from the standpoint of crossing-over, but there is no indication of its having occurred.

According to these results the genetic compositions of the three color types of Andalusians used in these experiments were as follows: blue-splashed = (*Re*)(*Re*), blue = (*Re*)(*rE*), and black = (*rE*)(*rE*). There was no evidence of crossing-over between *R* and *E* having occurred.

VI. Data from Crosses of Andalusians with Certain Recessive White Breeds

In the previous paper (1918*a*, p. 106) the writer reported a small number of data on a cross between a white Wyandotte ♂ and a blue-splashed Andalusian ♀. These have been considerably increased in amount and the reciprocal cross made. Further, both blue and black Andalusians have been crossed reciprocally with white Wyandottes and all three Andalusian color types crossed reciprocally with white Plymouth Rocks. The data from these several matings are set forth in Table II.

The crosses were made in the twelve possible ways, from eleven of which offspring were secured, the one type of mating which failed to produce offspring being the white Wyandotte ♂ × black Andalusian ♀. Inasmuch as

there is no evidence that any of the factors here under observation are sex-linked and there is considerable evidence that they are not, this omission is not serious.

TABLE II

SHOWING THE RESULTS OF CROSSING THE THREE-COLOR TYPES OF ANDALUSIANS WITH WHITE WYANDOTTES AND WHITE PLYMOUTH ROCKS

Group	♂♂	♀♀		Blue	Black
1.	Blue-splashed Andalusian *PP(Re)(Re)*	× white Wyandotte *pp(rE)(rE)*	Obtained	65	00
			Theoretical	65	00
2.	White Wyandotte *pp(rE)(rE)*	× blue-splashed Andalusian *PP(Re)(Re)*	Obtained	50	00
			Theoretical	50	00
3.	Blue-splashed Andalusian *PP(Re)(Re)*	× white Plymouth Rock *pp(rE)(rE)*	Obtained	179	00
			Theoretical	179	00
4.	White Plymouth Rock *pp(rE)(rE)*	× blue-splashed Andalusian *PP(Re)(Re)*	Obtained	87	00
			Theoretical	87	00
5.	Blue Andalusian *PP(Re)(rE)*	× white Wyandotte *pp(rE)(rE)*	Obtained	27	24
			Theoretical	25.5	25.5
6.	White Wyandotte *pp(rE)(rE)*	× blue Andalusian *PP(Re)(rE)*	Obtained	13	18
			Theoretical	15.5	15.5
7.	Blue Andalusian *PP(Re)(rE)*	× white Plymouth Rock *pp(rE)(rE)*	Obtained	80	55
			Theoretical	67.5	67.5
8.	White Plymouth Rock *pp(rE)(rE)*	× blue Andalusian *PP(Re)(rE)*	Obtained	24	32
			Theoretical	28	28
9.	Black Andalusian *PP(rE)(rE)*	× white Wyandotte *pp(rE)(rE)*	Obtained	00	18
			Theoretical	00	18
10.	Black Andalusian *PP(rE)(rE)*	× white Plymouth Rock *pp(rE)(rE)*	Obtained	00	132
			Theoretical	00	132
11.	White Plymouth Rock *pp(rE)(rE)*	× black Andalusian *PP(rE)(rE)*	Obtained	00	28
			Theoretical	00	28

The results of these crosses are understandable on the assumption suggested in the earlier paper that the individuals from the recessive white races are homozygous for the factors *E* and *p*, *p* being the recessive allelomorph of *P*, a factor necessary for the production of black pigment in the feathers. Sturtevant (1912) first suggested that Wyandotte white is recessive, a fact which was overlooked in my earlier paper. Morgan and Goodale (1912, p. 115) have made a similar assumption for the white Plymouth Rock.

Since in the series of experiments being reported here, reciprocal crosses of white Wyandottes and white Plymouth Rocks gave only whites, thereby showing no

evidence of recombination, it seems fair to assume that the white of both breeds is due to the same recessive factor *p* in homozygous condition.

The condition of the white Rocks and white Wyandottes reported in Table II, with reference to *E*, appears clear, since in all crosses with blue-splashed Andalusians (and as will appear later, in the case of the Wyandotte, with blue-splashed Orpingtons) which are homozygous for *P* and *R*, but do not carry *E*, all offspring, 381 in number, were without an exception, blue (see mating groups 1 to 4, Table II).

On this basis blue Andalusians, $PP(Re)(rE)$, mated with such recessive whites should produce blues and blacks in equal numbers. Mating groups 5 to 8, inclusive, in Table II show the results of such matings, which combined give 144 blues to 129 black (136.5 to 136.5 would be equality), a fair realization of the expectation.

As would be expected from the foregoing, crosses of similar recessive whites with black Andalusians ($PP(rE)(rE)$) (see Table II, groups 9 to 11, inclusive) gave only blacks. Of these there were in all 178 individuals and no exceptions.

The offspring of the crosses reported in Table II frequently gave evidence that the recessive white parents carried pattern factors as cryptomeres, but for the sake of clearness these complications, which have nothing directly to do with the study in hand, have been ignored in summarizing the data. As was to be expected, the white Plymouth Rocks carried the sex-linked pattern factor for barring. All pigmented offspring by a white Rock sire showed evidences of barring as soon as the definitive feathers appeared. Two such, the offspring of a white Plymouth Rock ♂ and blue Andalusian ♀ are shown in Fig. *G*, Plate II. Even at hatching, the occipital spot, which may be a juvenile effect of the factor for barring, gave notice of the presence of the barring factor. In the work here reported it was found possible to classify

in the down pigmented offspring of a non-barred ♂ × white Plymouth Rock ♀ cross accurately with regard to sex, by the presence or absence of the occipital spot. Morgan and Goodale (1912) made use of this spot in classifying barred and non-barred chicks which failed to hatch and Punnett (1919) also has made use of it in sorting newly hatched cross-bred chicks according to sex.

The progeny of crosses involving white Wyandottes frequently displayed Wyandotte lacing of a lesser or greater degree of perfection, though the appearance of this pattern was neither as constant nor as distinct as that of the barred pattern. The appearance of the lacing was to be expected if, as is generally stated in the literature on Wyandottes (see McGrew, 1901), the white variety was derived directly from the silver Wyandotte, which is laced.

In connection with these recessive white crosses is to be noted the fact that several white individuals, although "pure-bred" in the terminology of the poultryman, gave results which differed from the foregoing. Four white Wyandotte females proved to carry both the *R* and *E* factors and were of the same composition with respect to these factors as a pure-bred blue Andalusian, but unlike the blue Andalusian they carried *p* in the homozygous condition. One of these, which has already been reported on elsewhere (Lippincott, 1919), carried the sex-linked pattern factor for barring as well. Dryden (1916, p. 67) has also reported a white Wyandotte carrying a factor for barring.

One white Plymouth Rock and eight white Wyandottes proved to be heterozygous for a factor for dominant white. These were tested and found to be homozygous for *p*. In other words they carried both dominant and recessive white. Bateson and Punnett (1905, p. 117) appear to have had birds of this type and Dryden (1916, p. 66) reports a white Wyandotte which produced only white chicks when mated to a black Minorca, hence must

have been homozygous for a dominant white factor. Whether it carried P or p, the evidence does not show.

So far no attempt has been made to ascertain whether this factor for dominant white is the same as that normally carried by the white Leghorn and which Hadley (1913 and 1914) designated as I. For convenience and to recognize the possibility of its differing from I the factor here dealt with is referred to in this paper as I^P (inhibitor of pigment) and its allelomorph as i^P.

VII. Back-crosses of F_1's from Blue-splashed Andalusian × Recessive White Matings

The results of crossing the F_1 blues from the blue-splashed Andalusian × recessive white crosses is shown in Table III.

While by no means all possible back-crosses have been made, enough are represented to show clearly that factors R and E were appearing in approximately equal numbers, and that this was also true of P and p, though in some cases the presence of I^P complicated matters somewhat. It was, unfortunately, not always possible to use the actual parents in making back-crosses and though individuals from the same families were employed, this proved to be no criterion that they would be of the same genotype as the individuals used in the original cross. There can be no question as to their factorial composition, however, as each individual has been either deliberately tested or had happened to be so mated for another purpose as to give dependable evidence on its composition with respect to I^P and p.

So far as it goes, the evidence, which is substantiated by the results of other crosses to be reported in a later section of this paper, also shows that the meeting of P and R was according to chance, thereby indicating no linkage between these two factors.

It will be noted that the blue F_1 ♀♀ in group 5 of Table III had a blue Andalusian mother instead of a blue-

TABLE III

SHOWING THE RESULTS OF BACK-CROSSING F_1 BLUES FROM BLUE AND BLUE-SPLASHED ANDALUSIAN × RECESSIVE WHITE CROSSES

Group	♂♂	♀♀		Blue-Splashed	Blue	Black	White
1...	Blue F_1 $\frac{\text{spl. And. } ♂^3}{\text{wh. Wyand. } ♀}$ *Pp(Re)(rE)*	× blue-splashed Andalusian *PP(Re)(Re)*	Obtained	15	12	00	00
			Theoretical	13.5	13.5	00	00
2...	Blue F_1 $\frac{\text{wh. Wyand. } ♂}{\text{spl. And. } ♀}$ *Pp(Re)(rE)*	× blue-splashed Andalusian *PP(Re)(Re)*	Obtained	4	3	00	00
			Theoretical	3.5	3.5	00	00
3...	Blue F_1 $\frac{\text{spl. And. } ♂}{\text{wh. Rock } ♀}$ *Pp(Re)(rE)*	× blue-splashed Andalusian *PP(Re)(Re)*	Obtained	25	24	00	00
			Theoretical	24.5	24.5	00	00
4...	Blue F_1 $\frac{\text{wh. Wyand. } ♂}{\text{spl. And. } ♀}$ *PpiPiP(Re)(rE)*	× white Wyandotte *ppI^{Pi}P(rE)(rE)*	Obtained	00	12	7	64
			Theoretical	00	10.375	10.375	62.250
5...	White Plymouth Rock *pp(rE)(rE)*	× blue F_1 $\frac{\text{wh. Wyand. } ♂}{\text{blue And. } ♀}$ *Pp(Re)(rE)*	Obtained	00	8	8	24
			Theoretical	00	10	10	20
6...	White Wyandotte *ppI^{Pi}P(rE)(rE)*	× blue F_1 $\frac{\text{spl. And. } ♂}{\text{wh. Wyand. } ♀}$ *Ppi^{Pi}P(Re)(rE)*	Obtained	00	3	7	33
			Theoretical	00	5.375	5.375	31.250

[3] This convention in this and subsequent tables is used to indicate the kind and direction of the original cross.

splashed. From the nature of the behavior of the factors *R* and *E* already described, this would make no difference with regard to the blue offspring, for the blue progeny of a blue Andalusian female by a white Wyandotte male would be of exactly the same composition with respect to *R*, *E*, and *P* as *all* the offspring of a blue-splashed Andalusian mother by the same sire.

It will also be noted in this group (5) that while the father of the F_1 blue was a white Wyandotte, the male used in this cross was a white Plymouth Rock. Since it has been shown that for the factors being studied, white Plymouth Rocks and white Wyandottes are identical, this should not affect the ratios.

VIII. The F_2 Ratios from Blue-splashed Andalusian × Recessive White Matings

The F_2 ratios from various blue-splashed Andalusian × recessive white crosses are shown in Table IV.

As will be seen, the four F_2 classes predicted for such crosses in the writer's earlier paper (1918*a*, p. 113) on the basis of the F_1 results, have been obtained. No other classes have appeared. This would seem to indicate that the factorial compositions of the blue-splashed Andalusians and white Wyandottes then proposed were correct and that the white Plymouth Rocks used were of the same composition with respect to the factors *R*, *E* and *P* as were the white Wyandottes.

Seven F_1 blue males were used in securing the F_2 ratios. The legband numbers of these males may be found in Table IV, in the column headed "Band No." The direction of the original cross is indicated for each male and for the group of females with which he was mated. The direction of the cross was the same for the males and the females in all cases but two. Males 296M and 258M were mated with females which were products of the same crosses, respectively, as they themselves (groups 2 and 7), and also with females from the reciprocal crosses (groups 3 and 8).

TABLE IV

SHOWING F_2 RATIOS FROM CROSSES OF BLUE-SPLASHED ANDALUSIANS AND WHITE WYANDOTTES AND WHITE PLYMOUTH ROCKS[4]

Group	♂	Band No.	♀♀		Blue	Blue-spl.	Black	White
1...	Blue F_1 $\frac{\text{spl. And. ♂}}{\text{wh. Wyand. ♀}}$ $X^2 = 4.5747$	86E	× blue F_1 $\frac{\text{spl. And. ♂}}{\text{wh. Wyand. ♀}}$ $P = .2099$	Obtained	100	46	65	64
				Theoretical	103.1250	51.5625	51.5625	68.7500
2...	Blue F_1 $\frac{\text{spl. And. ♂}}{\text{wh. Wyand. ♀}}$ $X^2 = 4.0998$	296M	× blue F_1 $\frac{\text{spl. And. ♂}}{\text{wh. Wyand. ♀}}$ $P = .252516$	Obtained	73	24	39	48
				Theoretical	69	34.5	34.5	46
3...	Blue F_1 $\frac{\text{spl. And. ♂}}{\text{wh. Wyand. ♀}}$ $X^2 = .8759$	296M	× blue F_1 $\frac{\text{wh. Wyand. ♂}}{\text{spl. And. ♀}}$ $P = .83592$	Obtained	45	17	20	27
				Theoretical	40.875	20.4375	20.4375	27.2500
4...	Blue F_1 $\frac{\text{wh. Wyand. ♂}}{\text{spl. And. ♀}}$ $X^2 = 5.7301$	66M	× blue F_1 $\frac{\text{wh. Wyand. ♂}}{\text{spl. And. ♀}}$ $P = .1278$	Obtained	19	14	7	6
				Theoretical	17.25	8.625	8.625	11.5
5...	Blue F_1 $\frac{\text{wh. Wyand. ♂}}{\text{spl. And. ♀}}$ $X^2 = 4.6871$	65E	× blue F_1 $\frac{\text{wh. Wyand. ♂}}{\text{spl. And. ♀}}$ $P = .1998$	Obtained	94	41	44	42
				Theoretical	82.875	41.4375	41.4375	55.2500
6...	Blue F_1 $\frac{\text{spl. And. ♂}}{\text{wh. Rock ♀}}$ $X^2 = 2.7349$	46E	× blue F_1 $\frac{\text{spl. And. ♂}}{\text{wh. Rock ♀}}$ $P = .4395$	Obtained	34	10	17	18
				Theoretical	29.625	14.8125	14.8125	19.75
7...	Blue F_1 $\frac{\text{spl. And. ♂}}{\text{wh. Rock ♀}}$ $X^2 = 6.7510$	258M	× blue F_1 $\frac{\text{spl. And. ♂}}{\text{wh. Rock ♀}}$ $P = .081786$	Obtained	51	26	39	51
				Theoretical	62.625	31.3125	31.3125	41.75
8...	Blue F_1 $\frac{\text{spl. And. ♂}}{\text{wh. Rock ♀}}$ $X^2 = 8.20$	258M	× blue F_1 $\frac{\text{wh. Rock ♂}}{\text{spl. And. ♀}}$ $P = .042668$	Obtained	14	3	3	1
				Theoretical	7.8750	3.9375	3.9375	5.25
	Total ratios for all crosses $X^2 = 8.6410$ $P = .0353$			Obtained	430	181	234	257
				Theoretical	413.25	206.625	206.625	275.500

[4] The formulæ of color types involved in these crosses are: blue-splashed Andalusian *PP* (*Re*) (*Re*), white Rocks and Wyandottes *pp*(*rE*)(*rE*), and F_1 blues *Pp*(*Re*)(*rE*).

As may be seen by inspection of Table IV, but one male (296M) gave a group of offspring (3) which was very close to expectation. The chances that as great a deviation as this one would appear as a result of random sampling are four to one. The mothers of this group were the product of a cross which was the reciprocal of that which produced their sire. The offspring of 296M when mated with females which were the product of the same cross as himself (group 2) gave a deviation so great that the chances against its appearing as a result of random sampling are three to one. The chances of the appearance of deviations as great as those shown by the offspring groups of the other males were as follows: 86E (group 1) one chance in a little less than five; 66M (group 4) one chance in about eight; 65E (group 5) one chance in approximately five; 46E (group 6) one chance in about two and a quarter; 258M (group 7) once in about twelve times with females from the same cross as he, and once in twenty-five when mated with females from a reciprocal cross (group 8).

It would be unusual, though not impossible, to have so many comparatively wide deviations from expectation simply as a result of random sampling.

If the genetic constitution of the F_1's was as has been previously postulated, and these were in fact all chance deviations, it would be highly probable that the lumping of all the data given in Table IV would approximate the calculated ratio fairly closely.

The lumped data are given at the bottom of Table IV. It will at once be seen that the goodness of fit as measured by P is poorer than the poorest constituent group, and would be probable, on the basis of random sampling, once in about twenty-eight times. It seems fairly clear that some disturbing force was operative.

The two possible causes of disturbance which present themselves are linkage and a differential viability of classes, or it might be a combination of the two.

Linkage between the two principal pairs of factors involved in the crosses, Pp and $(Re)(rE)$, may not be appealed to because the only possible linkage relation would produce results diametrically opposed to those with which we are confronted. Since according to our hypothesis the recessive white parents were in each case of the composition $pp(rE)(rE)$ and the blue-splashed Andalusian parent $PP(Re)(Re)$, it is evident that linkage would require the production of $p(rE)$ gametes by the F_1 blues, more often than $P(rE)$ gametes. And similarly the combination $P(Re)$ should also appear more often than $p(Re)$.

A complete linkage between these pairs of allelomorphs would result in an F_2 ratio of 1 blue-splashed and 2 blue to 1 white, the blacks not appearing. The tendency of even weak linkage would be to reduce the proportional number of blacks. This should be true irrespective of the direction of the cross. It would further be true, that unless crossing-over occurred in both sexes any linkage whatsoever would inhibit the production of F_2 blacks homozygous for P. As will be shown in a later section of this paper, however, F_2 blacks homozygous for P have been identified. Even a casual inspection of Table IV shows that a relative preponderance of blacks is a quite constant characteristic.

Crossing-over in the male fowl has been found by Goodale (1917) and in the male pigeon by Cole and Kelley (1919). The latter investigators definitely state that there is no crossing-over of sex-linked factors in the female pigeon. Goodale states that none had been observed in the female fowl, but that a definite test of the matter would be made later. So far as the writer is aware no further report has been made. It should perhaps be pointed out that so far only sex-linked factors have been dealt with, no autosomal linked groups in birds having so far been reported.

There are no F_2 data available from crosses where p and (Re) are found in one parent and P and (rE) in the

other. The F_1's from such a cross have been secured by mating an extracted white of the composition $pp(Re)(Re)$ with a black Andalusian, $PP(rE)(rE)$, which gave all blues. From these an attempt will be made to secure F_2's in considerable numbers. Back-crosses to the parental types will also be made. The F_2's should approximate the same ratios as appear in Table IV and also give some evidence on the second possible explanation of the persistent deviations about to be discussed.

The calculation of theoretical expectancies presupposes the equal viability of all phenotypic and genotypic classes. If for any reason the individuals of one or more of the obtained classes tend to be less viable than certain other classes, deviation from expectancy will occur if the lack of viability expresses itself prior to making the counts.

As has already been pointed out, the lumping of the data presented in Table IV brings forth a poorer fit than is shown in any of the constituent groups. The deficient classes are the blue-splashed and the white, while the most preponderant class relatively is the black.

It seems to be a rather tacit assumption among poultrymen, particularly, it must in truth be said, among those breeding pigmented varieties, that the recessive white varieties are less vigorous (and so in all probability less viable) than the pigmented varieties of the same breeds. In how far this assumption is based on fact there is no critical evidence to call upon.

Regarding the relative viability of splashed and self-colored races there is no suggestion from any source. Splashed varieties are, so far as I am aware, nowhere bred as such, and the experience of practical breeders may accordingly not be appealed to.

While in the case in hand the assumption of low viability on the part of the individuals of the splashed and recessive white classes seems to correspond with the facts, such an assumption, though convenient, is not cor-

roborated by other evidence. That the splashed classes are not necessarily always deficient is shown by the progeny of the blue-splashed × blue mating in Table I, group 5, and of the F_1 blue × blue-splashed matings in Table III, groups 1, 2 and 3.

The latter fact suggests that possibly certain individuals used in these matings carried recessive factors tending to cause low viability, which were linked to the factor *R*. Until the fact of a differential viability is demonstrated, however, it is useless to speculate on this possibility. The reason for the deficiencies in the blue-splashed and also in the white classes, therefore can not at present be determined.

IX. Identification of the F_2 Genotypes

As indicated in my former paper (1918*a*, p. 113) the genotypes expected in the several F_2 phenotypes from the blue-splashed × recessive white crosses are as follows: blue, $PP(Re)(rE)$ and $Pp(Re)(rE)$; blue-splashed, $PP(Re)(Re)$ and $Pp(Re)(Re)$; black, $PP(rE)(rE)$ and $Pp(rE)(rE)$; white, $pp(Re)(Re)$, $pp(Re)(rE)$ and $pp(rE)(rE)$. Although the limitations of equipment were such that comparatively few F_2 individuals could be tested, fortunately all of the genotypes but one have been identified by making the appropriate crosses. The blues mated to individuals homozygous for *p* and *E* gave blues and blacks in equal numbers, or, blues, blacks and whites in the approximate ratio of 1:1:2, as the case might be. The blue-splashed mated to individuals of the same constitution produced all blues, or, equal numbers of blues and whites, depending upon whether or not they were homozygous with respect to *P*. Similarly the blacks gave all blacks, or, blacks and whites, depending upon their condition with respect to *P*.

The whites on the other hand were mated to blacks known to be homozygous for *P* and *E*. The $pp(Re)(Re)$ whites, as mentioned in an earlier section of this paper,

gave all blues, just as would blue-splashed Andalusians. The $pp(Re)(rE)$ whites produced blacks and blues in approximately equal numbers, exactly as would blue Andalusians. The parental white genotype $pp(rE)(rE)$, which would give all blacks, was curiously enough, the one of the whites which did not happen to be selected for testing.

It is important to note that while eight out of the nine F_2 genotypes were identified, no genotypes were found other than those expected.

X. Data on Andalusian × Black Langshan Crosses

It appeared desirable, in order to ascertain whether there was anything inherent in Andalusian black which made its relation to Andalusian blue different from that of other black breeds, to make certain matings of Andalusians with black Langshans. The Langshan was chosen because not only is it a different breed, but it also belongs to a different group of breeds. The original black Langshans were, according to Brown (1906, p. 63), imported from China, while the Andalusians, according to the same authority (p. 107), originated from native stocks along the borders of the Mediterranean Sea. So far as is known they have nothing in common in their immediate ancestry. Davenport (1914) even points to the probability that the immediate wild ancestors of the Asiatic breeds differed from those of the Mediterranean breeds. If blacks differ in their relation to Andalusian blue it would seem probable that Andalusian black and Langshan black might show this difference.

The results of the Andalusian-Langshan matings are shown in Table V. As may be seen readily by reference to this table the results are in every case precisely those which might be expected if a black Andalusian had been substituted for the black Langshan. So far as the principal factors under discussion are concerned it appears that the black Langshans used were identical in composi-

TABLE V

SHOWING THE RESULTS OF SEVERAL ANDALUSIAN × BLACK LANGSHAN CROSSES

Group	♂	♀♀		Blue Splashed	Blue	Black
1..	Blue Andalusian *PP(Re)(rE)*	× black Langshan	Obtained	0	34	31
		PP(rE)(rE)	Theoretical	0	32.50	32.50
2..	Blue Andalusian	× black	wh. Wyand. ♂ / black Lang. ♀	Obtained	0	15	21
	PP(Re)(rE)		*Pp(rE)(rE)*	Theoretical	0	18	18
3..	Blue-splashed Andalusian *PP(Re)(Re)*	× black Langshan *PP(rE)(rE)*		Obtained	0	65	0
				Theoretical	0	65	0
4..	Black Andalusian *PP(rE)(rE)*	× black Langshan *PP(rE)(rE)*		Obtained	0	0	11
				Theoretical	0	0	11
5..	Blue blue And. ♂ / black Lang. ♀ *PP(Re)(rE)*	× blue	blue And. ♂ / black Lang. ♀ *PP(Re)(rE)*	Obtained	4	9	5
				Theoretical	4.5	9	4.5
6..	Blue blue And. ♂ / black Lang. ♀ *PP(Re)(rE)*	× black	blue And. ♂ / black Lang. ♀ *PP(rE)(rE)*	Obtained	0	12	9
				Theoretical	0	10.5	10.5
7..	Blue blue And. ♂ / black Lang. ♀ *PP(Re)(rE)*	× black Langshan *PP(rE)(rE)*		Obtained	0	12	13
				Theoretical	0	12.5	12.5
8..	Blue blue And. ♂ / black Lang. ♀ *PP(Re)(rE)*	× blue Andalusian *PP(Re)(rE)*		Obtained	5	6	2
				Theoretical	3.25	6.5	3.25

tion with the black Andalusians, being *PP(rE)(rE)*. The condition of the Langshan with respect to *P* was found by mating individuals with white Wyandottes, whereby only black, *i.e.*, pigmented, offspring were produced.

XI. THE RELATION OF ORPINGTON BLUE TO ANDALUSIAN BLUE

Among the Orpingtons, an English breed, is a blue variety. Like the blue Andalusian it is an inconstant breeder with regard to color, segregating into blue-splashed and blacks as well as blues. Though by no means as widely bred as the blue Andalusians, it has numerous admirers, some of whom have claimed verbally to the writer that the proportion of wasters, *i.e.*, blue-splashed and blacks, was much smaller than in the Andalusians, though no figures are obtainable by way of sub-

TABLE VI

SHOWING THE RESULTS OF CERTAIN BLUE, BLUE-SPLASHED AND BLACK ORPINGTON CROSSES AMONG THEMSELVES AND WITH OTHER BREEDS

Group	♂	♀♀		Blue-spl.	Blue	Black	White
1...	Blue Andalusian *(Re)(rE)*	× blue Orpington *(Re)(rE)*	Obtained	13	30	12	0
			Theoretical	13.75	27.50	13.75	0
2...	Blue-splashed Andalusian *(Re)(Re)*	× black Orpington *(rE)(rE)*	Obtained	0	37	0	0
			Theoretical	0	37	0	0
3...	Black Andalusian *(rE)(rE)*	× blue-splashed Orpington *(Re)(Re)*	Obtained	0	14	0	0
			Theoretica	0	14	0	0
4...	White Wyandotte *pp(rE)(rE)*	× blue-splashed Orpington *PP(Re)(Re)*	Obtained	0	21	0	0
			Theoretical	0	21	0	0
5...	Blue Orpington *(Re)(rE)*	× blue Orpington *(Re)(rE)*	Obtained	19	58	15	0
			Theoretical	23	46	23	0
6...	Blue F_1 wh. Wyand. ♂ / spl. Orp. ♀ *Pp(Re)(rE)*	× blue F_1 wh. Wyand. ♂ / spl. Orp. ♀ *Pp(Re)(rE)*	Obtained	60	26	24	25
			Theoretical	50.6250	25.3125	25.3125	33.7500
	$X^2 = 4.0505$ P = .2569						

stantiation. It seemed desirable from several standpoints to ascertain what factors were involved in the production of Orpington blue, and whether the blue Orpington differed from the blue Andalusian in its genetic behavior. A number of matings were accordingly made, the data from which are shown in Table VI.

These data are consistent with the supposition that the factors involved in the production of Orpington blue are identical with those which produce Andalusian blue. The crossing of blue Andalusians and blue Orpingtons gave exactly the same sort of result as that obtained by mating blue Andalusians *inter se*, as shown by group 1. The blue-splashed Orpingtons mated with white Wyandottes gave only blues (group 4) just as did the blue-splashed Andalusians. And finally the F_2 ratio from white Wyandotte $\times$ blue-splashed Orpington crosses gave the same phenotypic classes as were obtained in the F_2 from the white Wyandotte $\times$ blue-splashed Andalusian cross, with a deviation from expectancy as great as would be probable once in four times. It is interesting to note that while the white class is deficient in this case, the blue-splashed class is not.

XII. Data from Blue Leghorn Crosses

In the spring of 1917 there appeared in the large pure-bred single comb white Leghorn flock of the Pabst Stock Farm at Oconomowoc, Wisconsin, two blue females. The flock was not pedigreed and nothing is known of the individual ancestors of these birds. They were of fair Leghorn type and were, as far as known, the offspring of pure-bred white Leghorn parents. Through the courtesy of Mr. Fred Pabst, and Dr. L. J. Cole of the University of Wisconsin, these individuals came into the hands of the writer and were entered on the records of the Department of Poultry Husbandry of the Kansas State Agricultural College as numbers 767 and 768.

Number 767 was a fairly even shade of medium to light

blue when received and showed some evidence of barring, though this was not very distinct. Number 768 was much lighter in shade than 767 and showed no evidence of barring. In contrast with ordinary blue she would, from a little distance, be mistaken for a white. The pigment granules in both cases were round.

The results of mating these birds in various ways are presented in Table VII. The numbers are rather small

TABLE VII

SHOWING THE BREEDING BEHAVIOR OF TWO BLUE LEGHORN FEMALES, WHEN MATED WITH VARIOUS MALES OF KNOWN FACTORIAL COMPOSITION

♂		♀		Blue Splashed	Blue	Black	White
White Leghorn 117M *IIPP(rE)(rE)*	×	767 *iiPP(Re)(rE)*	Obtained	0	0	0	5
			Theoretical[5]	0	0	0	5
White Leghorn 117M *IIPP(rE)(rE)*	×	768 *iiPP(Re)(rE)*	Obtained	0	0	0	11
			Theoretical	0	0	0	11
Blue Andalusian 78M *PP(Re)(rE)*	×	767 *PP(Re)(rE)*	Obtained	2	4	2	0
			Theoretical	2	4	2	0
Blue Andalusian 78M *PP(Re)(rE)*	×	768 *PP(Re)(rE)*	Obtained	3	7	4	0
			Theoretical	3.5	7	3.5	0
White Plymouth Rock 155M *pp(rE)(rE)*	×	767 *PP(Re)(rE)*	Obtained	0	20	24	0
			Theoretical	0	22	22	0
White Wyandotte 192M *I^Pi^Ppp(rE)(rE)*	×	768 *i^Pi^PPP(Re)(rE)*	Obtained	0	8	8	12
			Theoretical	0	7	7	14
Blue white Rock ♂ 155M blue Leghorn ♀ 767 *Pp(Re)(rE)*	255M ×	767 *PP(Re)(rE)*	Obtained	2	6	1	0
			Theoretical	2.25	4.50	2.25	0
Black Andalusian 288M *PP(rE)(rE)*	×	768 *PP(Re)(rE)*	Obtained	0	25	22	0
			Theoretical	0	23.5	23.5	0

but two facts seem fairly evident. First, that 767 and 768 are alike with respect to the factors under discussion in this paper, and second, that they give no indication of being different in their make-up with respect to the factors *R*, *E* and *P* from pure-bred blue Andalusians.

The appearance of the blue offspring of 768 (which it will be recalled was very light) when mated with black or blue Andalusians, was such as to suggest the possibility that accessory factors, necessary for the production of blue of normal shade, were supplied by the Anda-

[5] The theoretical expectancies calculated as for blue Andalusians.

lusian males, though no attempt was made to isolate and identify them.

Since these blue Leghorns arose in an unpedigreed flock, their origin is conjectural. A plausible explanation seems to be that two individuals heterozygous for *I*, the dominant Leghorn factor described by Hadley (1913), which inhibits the production of pigment shown (also by Hadley, 1914) to be normally present in the white Leghorn, happened to mate and that at least one of them carried the factor *R* as a cryptomere. That white Leghorns may sometimes carry the factor seems to be shown by the fact that Dryden (1916, p. 67) secured blue chicks in an F_2 generation from a barred Plymouth Rock $\times$ white Leghorn cross. And further, in the course of the breeding operations reported in this paper, blues appeared in the progeny of a black Andalusian and a white crossbred, the latter being the product of a black Andalusian $\times$ white Leghorn cross. In both cases it appears that the factor *R* must have been brought in by the white Leghorn. This suggestion also involves the assumption that the white Leghorn carries the factor *E*. That this is the case is shown by the fact that in the F_2 from a blue-splashed Andalusian $\times$ white Leghorn cross, the details of which are reserved for later publication, both blacks and blues appeared.

XIII. The Problem of True-breeding Blues

The fact that the blue varieties of both Andalusians and Orpingtons as now constituted do not breed true is a matter of considerable importance to their breeders. It is a heavy handicap to both varieties. While one hundred per cent. of blues may in each case be secured by mating blue-splashed individuals with black, as a matter of practical breeding this mating is seldom made. This is owing to the fact that there are several more or less variable qualities of color for which rigid selection is practised which are not apparent in either the blue-

splashed or blacks. The breeder therefore prefers to use for breeding purposes only those individuals which show the desired phenotypic condition, even though so doing necessitates the discarding of approximately half the offspring. While this leaves a comparatively small number of individuals, as compared with other breeds, upon which to practise selection, the blue Andalusian at least is bred in considerable numbers, thereby indicating its economic desirability and its attractiveness.

As was pointed out in the earlier paper (1918, p. 111) if *R* and *E* are not at identical loci on homologous chromosomes and crossover individuals were found which produce *RE* gametes, the problem of the constant-breeding blue would be solved.

The situation regarding black in rats may not be without its bearing in the present case. Black rats which bred true have been known for some time. Castle (1919) has, however, reported certain races of blacks which failed to breed true. This type of black was tested through several generations by Castle (1919), Ibsen (1920) and Dunn (1920). Blacks mated to blacks quite persistently produced whites, blacks and red-eyed yellows in the ratio of 1 to 2 to 1. Castle (1919) found one possible cross-over individual which died without being tested. Ibsen (1920) has so far failed to find any, and Dunn (1920) reports between one and two per cent. of cross-overs. These cross-overs, which were longer sought for and among larger numbers than has yet been possible with Andalusians, would appear to make it possible to synthesize a true breeding (*i.e.*, homozygous) black, from the line which has not been breeding true through a considerable number of generations.

It is also worth noting in this connection the possible bearing of Sturtevant's (1919) finding families of *Drosophila* carrying at least two definite factors in the second chromosome which almost completely inhibit crossing-over in the region contiguous to their loci.

If, however, after a long-continued search, it becomes increasingly evident that *R* and *E* are indeed allelomorphs, as originally suggested by Bateson and Punnett (1905), it was suggested (p. 113) that hope might be seen in the progressive selection of the darker, that is, more fully pigmented, blue-splashed individuals, there being considerable variation among the latter in this regard.

There is a further possibility which should not be overlooked, namely, that other factors might be found, perhaps in other breeds, which would act on black pigment to give the blue appearance on the one hand, or extend it to give self-colored individuals on the other. If duplicate factors for *E* or *R* should be found, a means of producing the long sought true-breeding blue would seem to be at hand. The fact that three factors are known which produce white in fowls lends emphasis to the possibility. It would seemingly make little difference in the ultimate outcome whether the new factor was linked to *R* and *E*, or was located on a different chromosome pair. In either case it would be possible to get a "self-coloring" and a "bluing" factor in the same gamete which, it appears, has so far not been done.

XIV. SUMMARY

1. It has been shown that the development of black pigment in the blue-splashed, blue and black races of the Andalusian and Orpington breeds, and of black Langshans, depends upon the action of a dominant hereditary factor *P*, for which they are normally homozygous.

2. The allelomorph of *P* is *p*. Individuals homozygous for *p* are white, as in the white Wyandotte and white Plymouth Rock breeds.

3. The extension of black pigment to all feathers of the body, resulting, if no pattern factors are present, in self-colored individuals, depends upon a dominant factor *E*. This factor has been found in the Andalusian, Orpington, white Plymouth Rock, white Wyandotte and black Lang-

shan breeds. Some evidence is presented which indicates its presence in white Leghorns.

4. The blue appearance of blue and blue-splashed Andalusians and Orpingtons, is due to the arrangement and restriction of black pigment, the result of a dominant factor *R*. This factor has also been found in individuals of the white Wyandotte and white Leghorn breeds, though its presence is probably not usual in these breeds.

5. No individuals of the Andalusian, Orpington, white Plymouth Rock, white Wyandotte or black Langshan breeds have been found which did not carry *R*, *E* or both.

6. The mutual relations of *R* and *E* are such that they have never been found together in the same gamete. This indicates that they are allelomorphic, *i.e.*, occupy identical loci on homologous chromosomes, or, each is so closely linked to the recessive allelomorph of the other, (Re) and (rE), that crossing-over rarely, if ever, occurs.

7. No evidence of crossing-over between *R* and *E* has been found and the tentative conclusion must be in accord with that previously held, that *R* and *E* are allelomorphs.

8. Both *R* and *E* are independent of *P* in their hereditary behavior, though dependent upon its presence for their manifestation.

9. The cooperative influence of the ovary is necessary for a full expression of *R* in the regions of the neck, back and saddle.

10. On the basis of the evidence presented in the body of this paper the genetic formulæ of the breeds and varieties employed, with respect to the factors under observation, are usually as follows: blue-splashed Andalusians and Orpingtons $PP(Re)(Re)$; blue Andalusians and Orpingtons $PP(Re)(rE)$; black Andalusians, Orpingtons and Langshans $PP(rE)(rE)$; and white Plymouth Rocks and Wyandottes $pp(rE)(rE)$.

11. The possibility of the occurrence of factors which duplicate the somatic effects of *R* and *E* is pointed out, and the relation of this possibility to the production of constant-breeding blues briefly discussed.

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INHERITANCE IN NICOTIANA TABACUM. II. ON THE EXISTENCE OF GENETICALLY DISTINCT RED-FLOWERING VARIETIES[1]

DR. R. E. CLAUSEN AND DR. T. H. GOODSPEED
UNIVERSITY OF CALIFORNIA

In our studies of inheritance in *Nicotiana Tabacum*[2] it has been demonstrated that the red flower color of *macrophylla* (U. C. B. G. 22/07) is recessive to the light pink of *angustifolia* (U. C. B. G. 68/07), and the same relations are exhibited by the red of *calycina* (U. C. B. G. 110/05) as contrasted with the light pink of *virginica* (Maryland, U. C. B. G. 78/05). In both cases the F_1 was pink, F_2 conformed to the ratio 3 pink:1 red, and F_3 and subsequent generations yielded data consistent with a single factor difference between these two flower colors. It was also shown that when *macrophylla* was crossed with the white-flowering variety *alba* (U. C. B. G. 30/06), F_1 was pink, F_2 conformed to the ratio 9 pink:3 red:4 white, and F_3 and subsequent generations gave data in agreement with a two-factor difference for this character contrast.

Allard,[3] however, had presented evidence, at first sight contradictory to ours, to the effect that the carmine flower

[1] The experimental data cited herein were obtained from cultures made possible by a portion of the Adams' Fund allotted to the Department of Botany by the Department of Agriculture of the University of California.

[2] Setchell, W. A., T. H. Goodspeed and R. E. Clausen, "A Preliminary Note on the Results of Crossing Certain Varieties of *Nicotiana Tabacum*," *Proc. Nat. Acad. Sci.*, 7: 50–56, 1921. A complete illustrated account of these experiments is in press under the title, "Inheritance in *Nicotiana Tabacum*. I. A Report on the Results of Crossing Certain Varieties," *Univ. Calif. Publ. Botany* 5, no. 17. For descriptions and illustrations of the varieties mentioned in this paper cf. Setchell, W. A., "Studies in *Nicotiana*. I., *Univ. Calif. Publ. Botany*, 5: 1–86, 1912.

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color of his Giant Red-flowering tobacco is dominant to pink in a simple mono-hybrid relation, F_1 being carmine and F_2 3 carmine : 1 pink. He also crossed this carmine-flowering variety with a white-flowering form and obtained light carmine in F_1 and a distribution which might be taken to conform to the ratio 9 carmine : 3 pink : 4 white. We took these results to indicate that his carmine, which must be very similar to our red, was nevertheless genetically distinct from it. This belief was somewhat strengthened by the fact that our red does not fall upon the carmine of the Ridgway[4] color scale, but lies slightly removed from it between rose red and pomegranate purple, although a difference of this kind might conceivably be due to the effect of differences in the residual genotype. We have, however, a variety, *purpurea* (U. C. B. G. 25/06), which exhibits a red flower color somewhat darker and more intense than that of *macrophylla*, and which some preliminary crosses indicated was dominant to pink and white. We accordingly suggested the following factor formulæ for these four colors:

WWRRPP = carmine
WWRRpp = light pink
WWrrpp = red
wwRRpp = white

In this formulation *WWRRPP*, represents the basic type, carmine in color; *w*, the difference from it which gives white, irrespective of which members of the pairs occupy the *R* or *P* loci; *p*, that which gives pink; and *r*, that which changes pink to red. Obviously white-flowering varieties may be of four different genotypes, viz., *wwRRPP*, *wwRRpp*, *wwrrPP*, and *wwrrpp*, but our white variety *alba* was clearly *wwRRpp*. This formulation brings our results into accord with those of Allard and accounts for the existence of genetically distinct red-flowering varieties. We have now obtained further evidence in support of the correctness of this formulation.

[4] Ridgway, R., "Color Standards and Color Nomenclature," 1912.

We found it necessary to use "Cuba" (U. C. B. G. 200/14),[5] another white-flowering variety, in these studies. Since there is the possibility just indicated of the existence of genetically distinct white-flowering varieties, it became necessary to determine the genetic constitution of "Cuba" with respect to the *Rr* and the *Pp* pairs of allelomorphs. A number of crosses were made, therefore, between "Cuba" and *macrophylla* as the starting point for these determinations. In the account which follows H_{174} = *macrophylla* ♀ × "Cuba" ♂ and H_{175} = the reciprocal. In the season of 1919, 50 plants of F_1H_{174} and 100 plants of F_1H_{175} were grown. They were all pink-flowering except that one plant produced a small white-flowering branch in an inflorescence otherwise pink-flowering. This bud variant, one of the few which we have observed in tobacco, will be taken up in a subsequent report. The further data on these reciprocal hybrids are listed in Table I. The F_2 popula-

TABLE I

F_2 AND BACK-CROSS DATA OF THE CUBA-MACROPHYLLA (WHITE × RED) SERIES

Garden Numbers	Parentage	Flower Color			Totals
		Pink	Red	White	
20.075....	$19F_1H_{174}P_{28}W$	59	22	19	100
20.076....	$19F_1H_{174}P_{28}P$	54	22	23	99
Totals for F_2 populations		113	44	42	199
$16F_1H_{182}$..	$15F_1H_{174}$ ♀ × 200/14 ♂	12	—	12	24
$19F_1H_{182}$..	ditto	48	—	50	98
$16F_1H_{183}$..	200/14 ♀ × $15F_1H_{174}$ ♂	12	—	13	25
$19F_1H_{183}$..	ditto	53	—	47	100
$16F_1H_{188}$..	200/14 ♀ × $15F_1H_{175}$ ♂	11	—	13	24
$19F_1H_{188}$..	ditto	50	—	49	99
$16F_1H_{189}$..	$15F_1H_{175}$ ♀ × 200/14 ♂	6	—	19	25
$19F_1H_{189}$..	ditto	53	—	47	100
Totals for back-crosses to white		245	—	250	495
20.059....	$19F_1H_{174}$ ♀ × 22/07 ♂	22	28	—	50

[5] For description cf. Goodspeed, T. H. "Parthenogenesis, Parthenocarpy and Phenospermy in *Nicotiana*," *Univ. Calif. Publ. Botany*, 5: 249–272, 1915.

tions give totals of 113 pink:44 red:42 white, whilst the 9:3:4 expectation, disregarding fractions, is 112 pink:37 red:50 white. In the back-crosses to both the white and the red parents the data are obviously in satisfactory agreement with the 1:1 expectations. These figures do not establish conclusively the validity of a bigenic formulation for this case, but taken together with the data from the ALBA-MACROPHYLLA series which we have presented elsewhere[6] it seems most reasonable to interpret them in this manner. An alternative mono-hybrid interpretation might be argued, but it would not fit the F_2 totals as well as the dihybrid ratio. The growing of F_3 populations would, of course, soon settle the question, but the results so far secured indicate essential genetical identity of *alba* and "Cuba" in their flower color factors.

In order to demonstrate the difference in behavior of red of *macrophylla* and carmine of *purpurea* we have made parallel crosses between them and a number of other *Tabacum* varieties. The flower colors of these varieties and of their F_1 hybrids with *macrophylla* and *purpurea* are listed in Table II. In each case the F_1 with *macrophylla* was pink but with *purpurea* it was always a full, intense carmine. Among two hundred plants of the CUBA-PURPUREA series one plant appeared which bore

TABLE II

F_1 RESULTS OF PARALLEL CROSSES OF MACROPHYLLA AND PURPUREA WITH A SERIES OF TABACUM VARIETIES

Variety Name and Number	Flower Color	Flower Color of F_1 with Macrophylla	Flower Color of F_1 with Purpurea
angustifolia (U. C. B. G. 68/07)	Light pink	Pink	Carmine
"Cavala" (U. C. B. G. 72/05)	Pinkish	Pink	Carmine
"Cuba" (U. C. B. G. 200/14)	White	Pink	Carmine

carmine flowers on one side and light pink ones on the other. Further studies on this, the most striking case

[6] Setchell, Goodspeed, and Clausen, *loc. cit.*

of somatic variation we have ever observed in *Nicotiana*, are in progress. The F_1 results in themselves sufficiently demonstrate the existence of a genetic difference between the red of *macrophylla* and the carmine of *purpurea*.

We have also secured further data from the CUBA-PURPUREA series which demonstrates the mode of inheritance of carmine when crossed with the same white used in the CUBA-MACROPHYLLA series. These results are set forth in Table III. The totals from the F_2 populations,

TABLE III

F_2 AND BACK-CROSS DATA FOR THE CUBA-PURPUREA (WHITE × CARMINE) SERIES

Garden Numbers	Parentage	Flower Color			Totals
		Carmine	Pink	White	
$19F_2H_{190}$..	$16F_1H_{190}P_8$	58	14	26	98
$19F_2H_{191}$..	$16F_1H_{191}P_6$	28	8	11	47
20.077....	$19F_1H_{191}P_4R$	48	13	39	100
20.078....	$19F_1H_{191}P_4P$	56	13	31	100
Totals for F_2 populations		190	48	107	345
20.060....	200/14 ♀ × $19F_1H_{191}P_4R$ ♂	16	6	28	50
20.061....	200/14 ♀ × $19F_1H_{191}P_4P$ ♂	12	12	25	49
Totals for back-crosses to white		28	18	53	99

190 carmine : 48 pink : 107 white, are to be compared with a 9 : 3 : 4 expectation of 194 carmine : 65 pink : 86 white. The results from the back-crosses, 28 carmine : 18 pink : 53 white, are to be compared with an expectation based on the 1 : 1 : 2 ratio of 25 carmine : 25 pink : 49 white. Pink is again deficient and white in excess, but not to such an extent as to give significance to the figures. Further data from F_3 families would be desirable for completion of the analysis. Thus far the data are in agreement with those presented by Allard for carmine versus pink and white, and they support the conclusion that his carmine variety is identical in its main genetic flower color factors with ours.

The further question now arises as to whether there are any phenotypic differences between carmine and red. There is a detectable difference between the flower color of *macrophylla* and that of *purpurea*, for the former has distinctly more of a purplish tinge and is not quite as intense in coloration as the latter. But these two varieties differ genetically in a large number of other characters. It is not possible, therefore, to decide the question by direct examination, because any distinctions which are found to exist may depend upon differences in the residual genotype rather than upon the specific factor differences which we have studied. Obviously the most satisfactory material for determining the differences between the two colors would be two varieties which had the same residual genotype, but the establishment of such varieties would entail the expenditure of a considerable amount of time and labor. We can, however, obtain some evidence on this problem by comparing the red F_2 segregants of the CUBA-MACROPHYLLA series with the carmine ones from the CUBA-PURPUREA series. In both cases there was a certain amount of variation in intensity of coloration in the F_2 classes, but it was found that, if they were mixed together, it was impossible to separate them again into red and carmine. In cases involving both classes in the same experiment, they would doubtless have to be considered as making up a single phenotype.

We have been interested in determining experimentally whether the morphological similiarities of existing *Tabacum* varieties might safely be taken as an index of phylogenetic affinities. Thus Setchell,[7] commenting on the relationships of *purpurea*, states,

> There are combined in this plant characters of our *N. angustifolia* as to petiole, *N. Tabacum* var. *brasiliensis* as to cucullate tip, tallness, and perhaps also the wing on the petiole, and *N. Tabacum* var. *macrophylla* as to flowers.

It is very natural to regard the sharply constricted leaf-

[7] *Loc. cit.*, p. 11.

base of *purpurea* as a modified petiolate condition, but as a matter of fact our studies have shown that its affinities in this respect lie closer to the sessile leaf type gentically, to which it is recessive, than to the true petiolate class which is dominant to the sessile type. In the present article we show further that the flower colors of *macrophylla* and *purpurea* are distinctly different genetically and their similarity in appearance can not be regarded as an indication of phylogenetic relationship. It is, therefore, evident that any taxonomic system which proposes to portray the phylogenetic affinities of the polymorphic assemblage of *Tabacum* varieties must be derived from genetic studies of character differences.

Allard has suggested the use of these flower color forms for instructional work in genetics. The demonstration of these additional relations increases their interest and value for such purposes. Among other points of interest a cross between *macrophylla* and *purpurea* should give a carmine F_1 and the rather unusual F_2 segregation ratio of 13 carmine (and red):3 pink. We have verified the production of carmine F_1 in this cross, but have not yet grown the F_2 progeny. The ease of hybridization, the readiness with which large quantities of guarded seed may be secured, and the extremely long period over which the seed of tobacco retains its viability may be urged as additional advantages in its utilization. Where greenhouse and garden space is available for their growth—plants may easily be grown to maturity in six-inch pots—these varieties and their hybrids would provide excellent material for practise in hybridization and for demonstrations of segregation and unique character interrelations. While there is a certain amount of variation within the several phenotypes here considered, viz., carmine, red, pink, and white, it has not been found to interfere seriously with segregation into the main color classes.

AN ANALYSIS OF THE RELATION BETWEEN GROWTH AND NUCLEAR DIVISION IN A PARASITIC INFUSORION, OPALINA SP.[1]

R. W. HEGNER, PH.D.

SCHOOL OF HYGIENE AND PUBLIC HEALTH, JOHNS HOPKINS UNIVERSITY

AND

HSIANG-FONG WU, M.D.

NATIONAL MEDICAL COLLEGE OF PEKING, CHINA

THIS investigation was undertaken for the purpose of analyzing the relation between growth and nuclear division in a species of *Opalina* of the frog during the growth period in the tadpole. The multinucleate condition of *Opalina* and the absence of cell walls render it of particular value as material for the study of the phenomena involved in nuclear division and growth. The specimens used in our investigations were obtained by Dr. Charles E. Simon from tadpoles collected at Chester, Nova Scotia, during the summer of 1920. Unfortunately we are unable to state either the species name of the *Opalina* or that of the host. Dr. Maynard M. Metcalf, who has examined the slides, thinks the *Opalina* is probably an undescribed species. The material was well fixed in Schaudinn's solution and beautifully stained with iron-hematoxylin.

A sufficiently large number of specimens (455) were drawn with a camera lucida so as to furnish reliable results when measurements were treated by statistical methods. The area of the drawings was determined with a planimeter and the correlation with the nuclear number determined. Table I is the correlation table for the nuclear number and area of 341 specimens. The area of the drawings, which were made at a magnification of 650

[1] From the Department of Medical Zoology, School of Hygiene and Public Health, Johns Hopkins University.

diameters, is given in square millimeters. The coefficient of correlation is remarkably high, namely, $.755 \pm .016$; this proves that an increase in size is accompanied by a corresponding increase in nuclear number. The rest of the specimens that were measured, 114 in number, were drawn at a magnification of 1400 diameters. The coefficient of correlation of this lot was found to be $.875 \pm .015$.

TABLE I

CORRELATION TABLE FOR NUMBER OF NUCLEI AND AREA OF 341 SPECIMENS

The area is given in sq. mm. and obtained from camera-lucida drawings made at a magnification of 650 diameters. Coefficient of correlation $.755 \pm .016$.

Area	Number of Nuclei																	
	3	4	5	6	7	8	9	10	11	12	13	14	15	16	27	28	29	
300+		1																1
400+	1	1	2				1											5
500+		1	1	1	1													4
600+	1	7	7	3	1													19
700+		4	6	12	2													24
800+		2	12	13	5	3		1										36
900+			6	25	8	6	1											46
1000+			1	13	14	9	8											45
1100+		2		3	12	20	6	4	1									48
1200+			1	1	8	3	5	5	1									24
1300+	1	1	1	1	1	5	4	4	1		2							21
1400+				1	1	3	4	5	4	2								20
1500+				1				4	1	1	1	3						11
1600+							1	1	3	2	1		1					9
1700+			1		1		1	1							1			4
1800+			1						2	2					1			6
1900+			1	1		1	1		1	1	1							7
2000+													1					1
2100+									1	1		2						4
2200+									1									1
2400+								1					1	1				3
2800+																	1	1
3200+																1		1
	3	19	40	75	54	50	32	26	16	9	5	5	3	1	1	1	1	341

Metcalf[2] has pointed out that in multinucleate Opalinas the nuclei within a single specimen may be in different stages of division at one time. This we have found to be true also of the nuclei during the growth stages in the tadpole—a condition that has enabled us to analyze with

[2] Metcalf, M. M., 1909, "*Opalina*," *Arch. f. Protist.*, 13: 195–375. Especially p. 269.

considerable accuracy the exact relation between cytoplasmic mass and nuclear division. For example, among the specimens with four nuclei, there were a few with three "resting" nuclei and one nucleus in division (Fig. 3); obviously one of the four nuclei is undergoing division before its three sisters. If the sum of the areas of a number of specimens in which there are four nuclei of equal size (Fig. 2) is divided by the total number of nuclei, a fairly accurate idea may be obtained of the amount of cytoplasm associated with each nucleus. According to the nucleo-cytoplasmic relation theory[3] an increase in the amount of cytoplasm as compared with the amount of nuclear material furnishes the stimulus which initiates nuclear division. A comparison between specimens with four equal nuclei, and specimens with four nuclei one of which is undergoing division, should reveal approximately the increase of cytoplasmic substance necessary to inaugurate nuclear division. A number of cases of this sort were available in our material and were studied with the following results.

Table II shows the relations between area, and number, volume and surface of the nuclei in the 207 specimens that could be used for this purpose. The measurements were made of camera-lucida drawings at a magnification of 650 diameters. Beginning with the group of 15 at the top of the table we can make the following comparisons.

1. Fifteen specimens, each with 4 equal nuclei (Fig. 2), have an average area per nucleus of 176.1 sq. mm.; 10 specimens, each with 3 equal nuclei and a fourth nucleus in division (Fig. 3), have an average area per nucleus of 185.0 sq. mm. The specimens in which division has been initiated have an average area per nucleus 8.9 sq. mm. greater than those with an equal number of nuclei, none of which are in division. We have used the area through-

[3] For a recent discussion of this theory see Hegner, R. W. 1920, "Relations between Nuclear Number, Chromatin Mass, Cytoplasmic Mass, and Shell Characteristics in Four Species of the Genus *Arcella*," *Jour. Exp. Zool.*, 30: 1–95.

TABLE II

TABLE SHOWING THE RELATION BETWEEN AREA AND NUMBER, VOLUME AND SURFACE OF THE NUCLEI

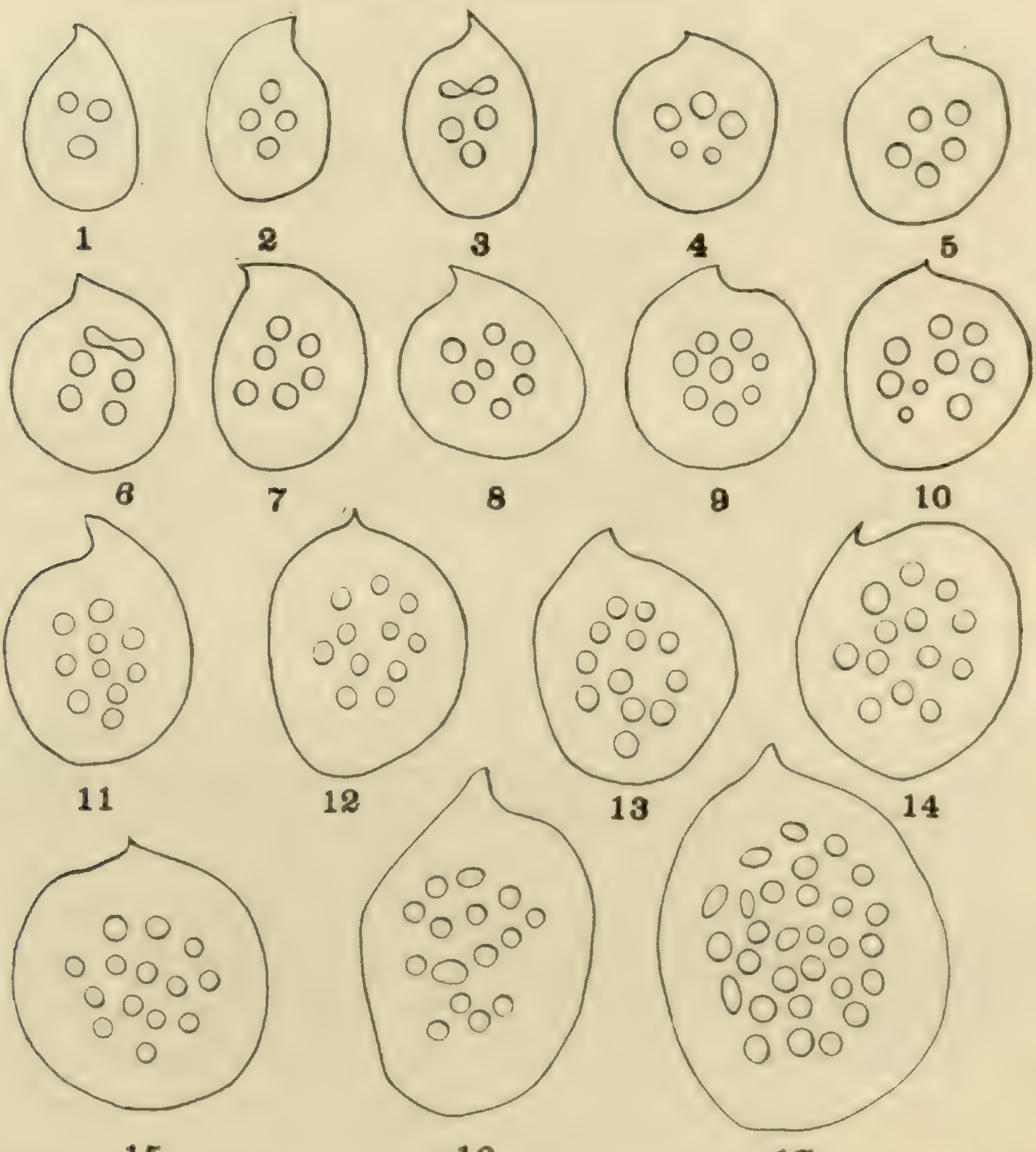
The area, volume and surface of the nuclei were computed from camera drawings made at a magnification of 650 diameters.

Number of Specimens	Number of Nuclei	Average Area in Sq. Mm. at Magnification of 650 Diameters	Average Area per Nucleus in Sq. Mm. at Magnification of 650 Diameters	Average Volume of Nuclei in Cubic Mm. at Magnification of 650 Diameters	Average Volume per Nucleus in Cubic Mm. at Magnification of 650 Diameters	Average Surface of Nuclei in Sq. Mm. at Magnification of 650 Diameters	Average Surface per Nucleus in Sq. Mm. at Magnification of 650 Diameters
15	4 (equal in size)	704.2	176.1	215.49	53.87	261.32	65.33
10...	4 (one in division)	739.9	185.0				
13...	5 (two small)	851.5	170.3	257.11	51.42	321.10	64.22
16...	5 (equal in size)	860.5	172.1	281.91	56.38	345.01	69.00
17...	6 (two small)	951.4	158.6	273.69	45.62	363.85	60.64
49...	6 (equal in size)	926.7	154.5	264.83	44.14	352.89	58.81
5...	6 (one in division)	1,032.5	172.1				
11...	7 (two small)	993.5	141.9				
31...	7 (equal in size)	1,066.7	152.4	269.17	38.45	379.68	54.24
10...	7 (one in division)	1,176.7	168.1				
30...	8 equal	1,127.9	141.0	336.53	42.07	458.93	57.37
207							

out our work as a measure of cytoplasmic mass, hence it appears from the results of our measurements that an increase in mass per nucleus represented by an increase in area within the limits of 8.9 sq. mm. is the stimulus that initiates nuclear division. The exact mass of cytoplasm represented by this increase in area of 8.9 sq. mm. might easily be obtained under more favorable circumstances.

2. When we compare the measurements of the 10 specimens with 4 nuclei, one of which is dividing (Fig. 3) with 13 specimens of the stage immediately following, with 3 large nuclei and two that have just reorganized after division (Fig. 4), we find that although the latter average 111.6 sq. mm. larger per specimen the average area per nucleus is 14.7 sq. mm. less. Thus there has been an

actual increase in size but a decrease in the mass of cytoplasm associated with each nucleus.



FIGS. 1–17. Outline drawings of stages in the growth of *Opalina* sp. made with a camera lucida at a magnification of 650 diameters and reduced to a magnification of 325 diameters.

3. During the growth of the two small nuclei to their full size (Figs. 4–5) the size of the organism increases from an average area of 851.5 sq. mm. per specimen to an average area of 860.5 sq. mm., or an average area per nucleus of from 170.3 sq. mm. to 172.1 sq. mm. Although an increase in size has taken place, the average area per nucleus of 172.1 sq. mm. in specimens containing 5 full-

sized nuclei is less than that of specimens with 4 full-sized nuclei, *i.e.*, 176.1 sq. mm.

4. A further increase in the average size of the specimens occurs between the stage with 5 nuclei of equal size (Fig. 5) and that with 6 nuclei, two of which have just emerged from mitosis. Measurements give for the former an average area of 860.5 sq. mm. and for the latter 951.4 sq. mm. The specimens with the two small nuclei, however, possess as before (see (2)) a lower average area per nucleus, *i.e.*, 158.6 sq. mm. as compared with 172.1 sq. mm. in specimens with 5 full-grown nuclei.

5. The measurements of the next stage, *i.e.*, specimens with 6 nuclei of equal size (Fig. 7), are more difficult to explain, since the average area of the specimens (926.7 sq. mm.) is actually less than that of the younger specimens (see (4)) with 4 large and 2 small nuclei, and the average area per nucleus falls from 158.6 sq. mm. to 154.5 sq. mm. These results may be due to a thickening of the entire animal which would increase the mass and tend toward a decrease in area or the nucleo-cytoplasmic relation may change as the animals become older. That there is an actual decrease in the average area per nucleus as growth proceeds is indicated by the measurements of later stages as given in Table III. This table shows a decrease per nuclear area from 186 sq. mm. in specimens with 4 nuclei to 96.8 sq. mm. in specimens with 29 nuclei. That this decrease is gradual is indicated when averages are made of three successive groups containing each a larger number of specimens. Thus the 4, 5, and 6 nucleated groups containing 134 specimens have an average area per nucleus of 173.8 sq. mm., the 7, 8, and 9 nucleated groups containing 136 specimens have an average area per nucleus of 144.4 sq. mm., the 10, 11, and 12 nucleated groups containing 51 specimens have an average area per nucleus of 143.5 sq. mm., and the 13, 14, and 15 nucleated groups containing 13 specimens have an average area per nucleus of 128.3 sq. mm.

TABLE III

TABLE GIVING THE AVERAGE AREA PER SPECIMEN AND PER NUCLEUS OF 338 SPECIMENS DRAWN WITH A CAMERA LUCIDA AT A MAGNIFICATION OF 650 DIAMETERS

Number of Specimens.	Number of Nuclei.	Average Area per Specimen in Sq. Mm. at Magnification of 650 Diameters.	Average Area per Nucleus in Sq. Mm. at Magnification of 650 Diameters.
19.....	4	744	186.0
40.....	5	891	178.2
75.....	6	944	157.3
54.....	7	1,069	152.7
50.....	8	1,150	143.7
32.....	9	1,231	136.8
26.....	10	1,395	139.5
16.....	11	1,635	148.6
9.....	12	1,709	142.4
5.....	13	1,572	120.9
5.....	14	1,801	128.6
3.....	15	2,032	135.4
1.....	16	2,452	153.2
1.....	17	1,806	106.2
1.....	28	3,277	117.0
1.....	29	2,806	96.8

6. By the time one of the nuclei of the six-nucleated stage has been stimulated to division the average area per nucleus increases again to 172.1 sq. mm. After this division is completed and seven nuclei are present, two of them small, the average area per nucleus, as was to be expected, decreased to 141.9 sq. mm. During the period necessary for the two small nuclei to reach their full size (Fig. 8) the area increases again to 152.4 sq. mm. A further increase to 168.1 sq. mm. occurs by the time sufficient growth takes place to stimulate one of these seven nuclei to divide, and a decrease (to 141.0 sq. mm.) again takes place when this stage evolves into that with eight nuclei (Fig. 9).

The two curves in Fig. 18 show clearly the increase in area per nucleus up to the point where one nucleus divides, then a conspicuous decrease following nuclear division, and subsequently an increase during the period when the nuclei resulting from division regain their full size, ending in a size at which the area per nucleus is approximately that present at the beginning.

Figure 19 illustrates the fact that the size of the entire specimens increases during nuclear multiplication and growth, but that the area per nucleus remains almost constant.

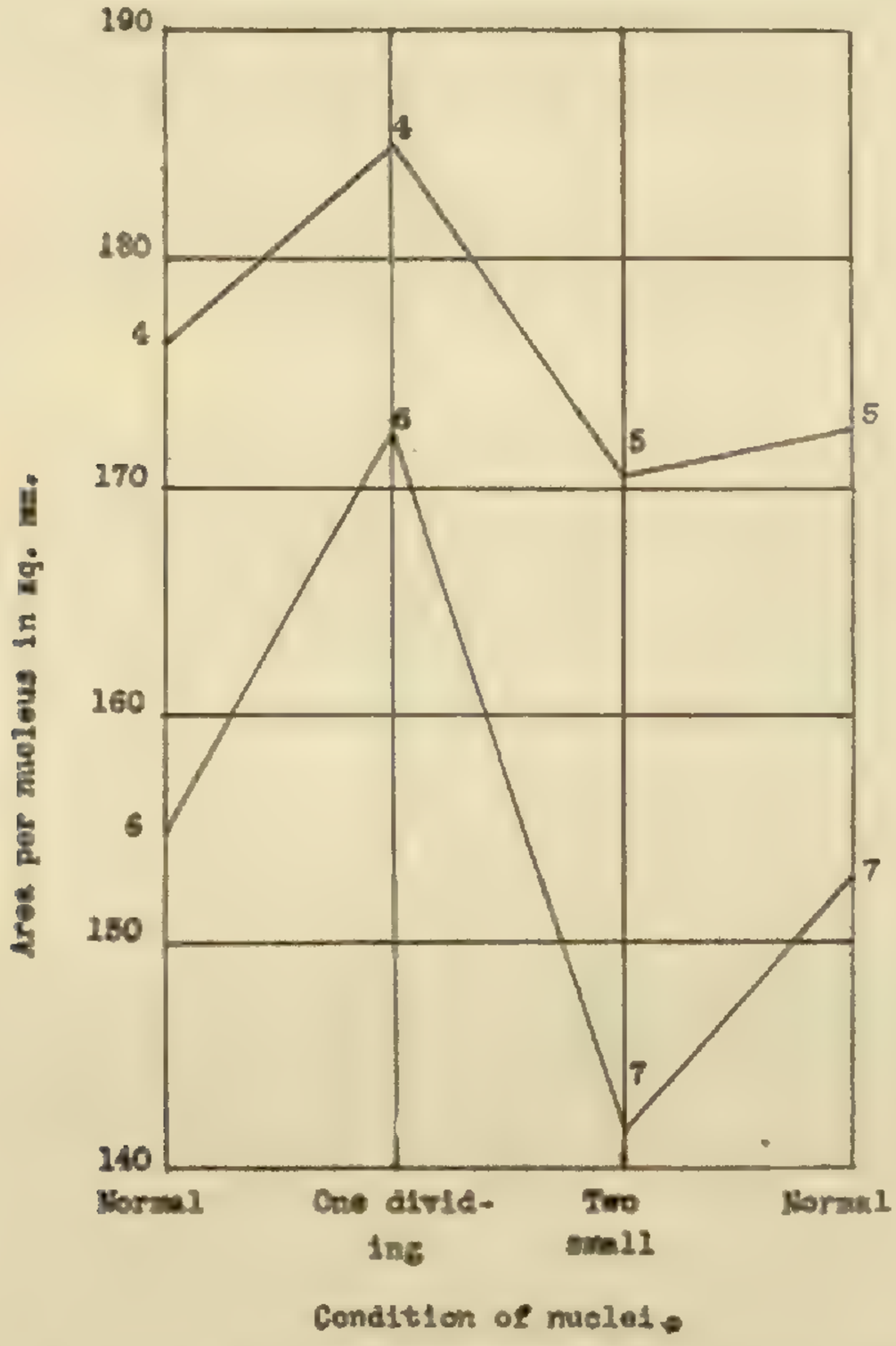


FIG. 18. Curves showing the changes in area per nucleus that accompany changes in nuclear number and condition. The numbers 4, 5, 6, and 7 indicate the number of nuclei present.

7. The series of measurements of these specimens affords an explanation of the reason why nuclear division in *Opalina* is not synchronous. According to the nucleo-cytoplasmic relation theory, as soon as the mass of cytoplasm has increased to a certain point nuclear division is initiated. The necessary increase to furnish this stimulus in *Opalina* may be determined approximately from our data by comparing measurements of specimens in which the nuclei are all equal in size with those in which nuclear division has been inaugurated. Such a comparison gives the following results.

Nuclei	Average Area per Nucleus	Difference in Area — Amount Necessary to Stimulate Division
4 (equal)	176.1 sq. mm.	
4 (one in division)	185.0 sq. mm.	8.9 sq. mm.
6 (equal)	154.5 sq. mm.	
6 (one in division)	172.1 sq. mm.	17.6 sq. mm.
7 (equal)	152.4 sq. mm.	
7 (one in division)	168.1 sq. mm.	15.7 sq. mm.

These figures, of course, indicate only the relative increase necessary to stimulate nuclear division; the actual increase could be determined by measuring accurately the mass of cytoplasm in each case.

FIG. 19. Curves showing the increase in the area of specimens with increase in age and the constancy of the area per nucleus.

In looking over our camera drawings it was noticed that usually only one nucleus was in division in any one specimen (Figs. 3 and 6) and that in many cases two of the nuclei in a specimen were smaller than the rest (Fig. 4), indicating that they were daughter nuclei that had just emerged from mitosis. Of a total of 137 specimens in which nuclei were found in division, 109 contained one division figure, 19 contained 2, 8 contained 3, and 1 contained 4. Furthermore, those containing more than

one division figure were usually older than those containing one only. Thus the average nuclear number of specimens with one dividing nucleus was 7.2, with two dividing nuclei, 8.1, with three dividing nuclei, 9.3, and with 4 dividing nuclei, 10. These data favor the conclusion that the stimulus that initiates nuclear division acts as a rule on only one nucleus at a time and that the division of this nucleus restores the nucleo-cytoplasmic ratio. When this ratio is again disturbed by an increase of the cytoplasmic mass another nucleus is stimulated to divide. Division of two or more nuclei synchronously may be due to the more rapid growth, the larger specimens in which this usually occurs, or to the greater chances of two or more nuclei reacting to the division-stimulus when a large number of nuclei are present in a single specimen. There is some evidence that the nucleus that undergoes division is the one with the greatest amount of cytoplasm surrounding it, but this could not be determined definitely. No regular distribution of the nuclei was evident. It is interesting to note in this connection that during the embryonic development of many animals nuclear division occurs in all cells at nearly the same time. This is especially interesting in the case of certain insects, in the eggs of which nuclear division proceeds synchronously without the intervention of cell walls until thousands of nuclei are present in a single egg.[4] An increase of cytoplasm over nucleus may also, in these insect eggs, stimulate nuclear division, since after each division the mass of cytoplasm surrounding each nucleus is increased by the addition of new material elaborated from the yolk substance in which it is situated.

8. The average total volume of the nuclei of certain specimens, average volume per nucleus, average total area of the surface of the nuclei and average area of the surface per nucleus were measured in cubic millimeters and square millimeters from our camera drawings which

[4] Hegner, R. W., 1914, "Studies on Germ Cells," *Jour. Morph.*, 25: 375–509. Especially pp. 408–413.

were magnified 650 diameters. These data are shown in Table II. They indicate one point of considerable interest and that is the fact that as the number of nuclei increases their average volume and surface decrease.

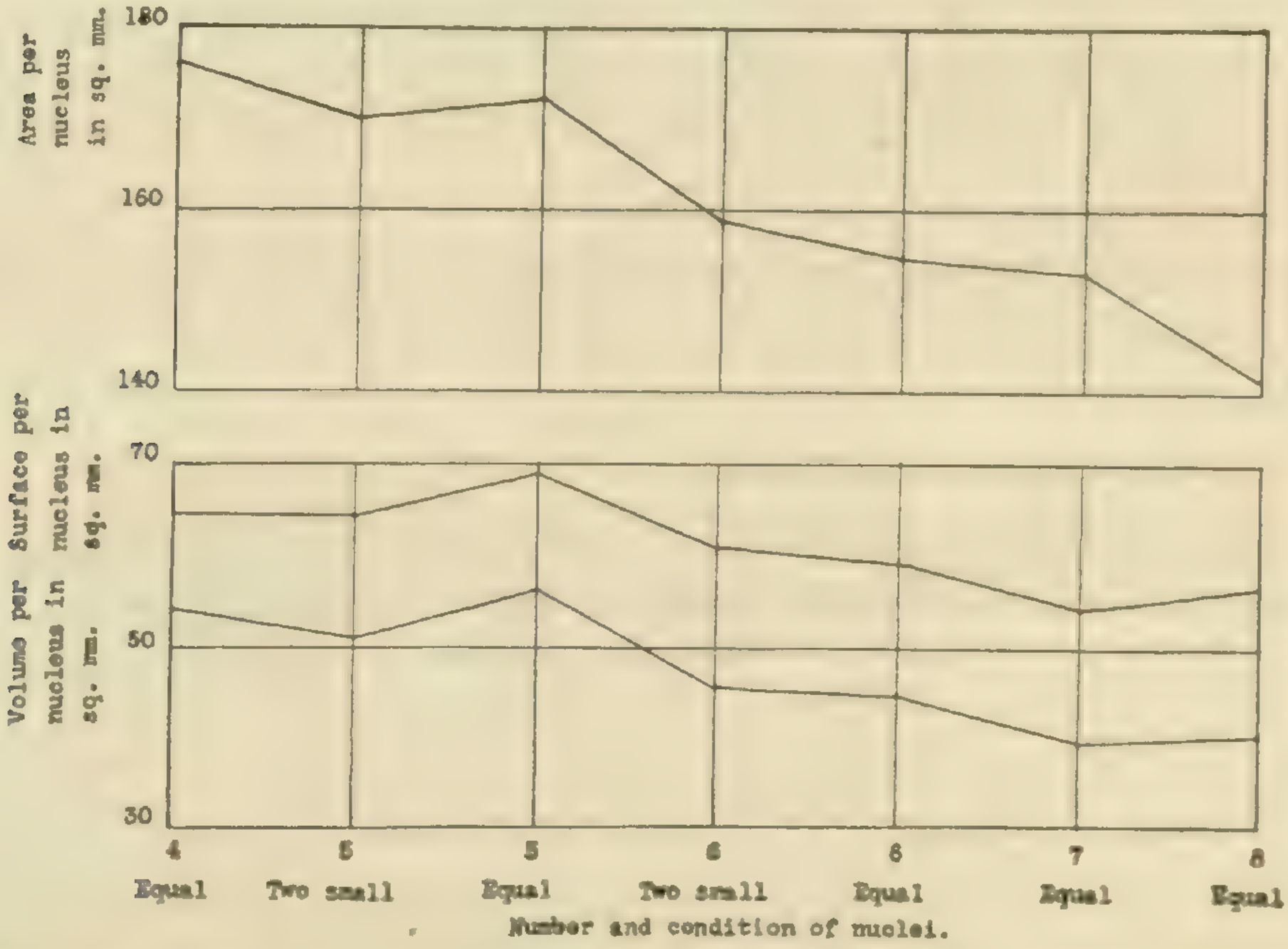


FIG. 20. These three curves show that decreases in the volume and surface of the nuclei are accompanied by decreases in area per nucleus.

Thus in specimens with 4 nuclei of equal size each nucleus has an average volume of 53.87 cu. mm., in specimens with 6 nuclei the average volume per nucleus decreases to 44.14 cu. mm., and in specimens with 8 nuclei the average volume per nucleus decreases still further to 42.07 cu. mm. Similar results were obtained from measurements of the surface of the nuclei, but the decrease is not so great since the volume decreases as the cube whereas the surface decreases only as the square. As the table (II) shows the average surface per nucleus in sq. mm. decreased from 65.33 sq. mm. in specimens with 4 nuclei, to 58.81 sq. mm. in specimens with 6 nuclei, and 57.37 sq. mm. in specimens with 8 nuclei. This decrease in volume and surface may account for the fact noted previously (in (5)), that the area per nucleus decreases in specimens with nuclei all equal in size as the number of

nuclei becomes greater with advancing age. Since the volume of the nuclei is less in these older specimens the amount of cytoplasm associated in normal nucleo-cytoplasmic relations with them is less and the area of the specimens per nucleus decreases accordingly.

The curves in Fig. 20 bring out clearly the relation between area and volume and surface of the nuclei during the growth period. The average area per nucleus decreases as the number of nuclei increases, but at the same time there is a corresponding decrease in both volume and surface of the nuclei, thus maintaining approximately the initial relation between nucleus and cytoplasm.

SUMMARY

(*a*) A high correlation exists between nuclear number and cytoplasmic mass (as indicated by area) during the growth of *Opalina* sp. The coefficient of correlation in one lot of 341 specimens was $.755 \pm .016$ and in another lot of 144 specimens was $.874 \pm .015$.

(*b*) By comparing the area of various stages with the number, size, state of division, volume and surface of the nuclei the following conclusions were reached. (1) Nuclear division is stimulated by an increase of cytoplasm that may be determined approximately. (2) As the organisms increase in age the nuclei decrease in volume and surface; this is accompanied by a corresponding decrease in the area per nucleus, indicating that the nucleo-cytoplasmic relation is maintained. (3) Nuclear division is not synchronous because one nucleus is usually stimulated to divide before the others, and this division is sufficient for the time to reestablish the normal relation between nuclei and cytoplasm.

AMERICAN FOLLICULINAS: TAXONOMIC NOTES

E. A. ANDREWS

THE JOHNS HOPKINS UNIVERSITY

THE ciliated infusorian *Folliculina*, "the bottle animalcule," was first recorded by O. F. Müller in 1781 and was by him described amongst 75 Vorticellas as one living in an ampulla or bottle. The name *Folliculina* was suggested by Lamarck in 1816, yet he, having no personal observations of the creature, placed it among the rotifers. However, the true affinities of the bottle makers with the stentors became evident to Claparede in 1858, though members of the group have been referred by others to such genera as *Cothurnia, Ascobius* and *Vaginicola* from insufficient knowledge of the animal within the bottle. Two observers emphasized the nature of the bottle *maker* rather than the bottle itself in seeking to establish for it the generic names Freia and Lagotia; the former given it by Claparede as having the "forme gracieuse et elegante d'une Freia," and the latter by Wright, in the same year, from the long lobes of the animal that resemble the ears of a hare.

The only careful studies of the animal have been made by Stein in 1867 and by Möbius in 1887. The latter was inclined to regard all the then known species as local varieties of the original *Vorticella ampulla* O. F. Müller. But recently Carl Dons in Norway has made very minute study of the bottles as found in many localities and has come to the conclusion that these alone may be used as sufficient basis for establishing species, even without the animal, which, to be sure, is rarely preserved in museum material. He would recognize some ten species, most of which he finds in Norway, but many of which are widespread over the world.

These ten species he proposes to distribute amongst four new genera as follows: The original forms of Müller

with simple bottles of very wide diameter and short plain neck with no lips retain the name *Folliculina*. The type species *F. ampulla* being found in Norway, Denmark, at Kiel and in the Adriatic, while a peculiar species *F. paguri* from the west French coast was described by Giard in 1880 as *Pebrilla paguri*.

Forms with more elongated bottles and with longer necks, often marked with a spiral ridge, as well as provided with a collar or lip to the mouth of the bottle, he calls *Semifolliculina*. The best described is his *S. gigantea* from Norway, as well as the South Polar Sea, while *S. boecki* is the name chosen for an old and widely distributed form occurring in Norway, Denmark, the Adriatic, North America, Formosa, as well as both North and South Polar Seas. *S. similis* is an aberrant form from the South Seas and *S. spirorbis* is the smallest form, with very narrow neck, found both in Norway and upon material from North America, East coast.

Long, straight bottles with the bottom thickened as a falsification of the actual content as judged from the outside are placed in the new genus *Pseudofolliculina*, represented by the large *P. mellita* from the South Polar Seas and by the similar species *P. arctica* from the north of Norway. The remaining bottle animals have been described as having some sort of a valve or set of membranes in the neck of the bottle, and these Dons includes in the genus *Parafolliculina*. *P. amphora* he has described minutely from Norway and from Iceland, and *P. violacea* is a well-known form from west France, the Adriatic, west Australia, as well as Norway.

All these bottles are minute, less than a millimeter long, and though made of a chitin-like material that admits of long preservation in museum jars, they have generally been overlooked, though so common in many parts of the world from the surface down to considerable depths attached to solid objects, such as shells, stones and plants, either singly or in large aggregates or settlements. Moreover, certain species have been described

in England, France and Switzerland as occurring in entirely fresh waters. The above review of the known species and their distribution does not do justice to Strethill Wright's careful description of the animals that make the cases or bottles on the British coasts where he found species not entirely synonymous with the above-cited ten.

The first record of the occurrence of any of these bottle animalcules along the American coasts seems to have been that of Leidy, who, in July, 1859, at Newport, Rhode Island, found attached to *Anomia-serpula* on dead clam shells dredged by Mr. Powel " a singular and beautiful animal " in a vase-like tube and with the same general structural appearance as that of the stentors.

He recognized its resemblance to *Chætospira mueleri* Lachman and its alliance with the stentors and suggested the name *Freyia Americana* for it. Later, according to Ryder, Leidy considered his species to be the same as the European *Folliculina ampulla.* It was not till 1880 that the bottle animalcule was again observed in American waters, and then Ryder on the western shore of the Chesapeake Bay, probably at St. Jerome, St. Mary's County, found a different form of bottle and animal which he identified with the *Freia producta* of Strethill Wright.

The occurrence of this form of bottle animalcule in other parts of the estuaries of the Chesapeake was pointed out in 1914, 1915 by Andrews under the name *Folliculina,* but without determination of the species described. Meantime it was known to workers at Woods Hole, Mass., that *Folliculina* occurred there also, though no published accounts appeared. Dons has recently mentioned the observation of a *Folliculina* upon material from the east coast of America and refers another species to this coast, probably from the above account of Leidy.

We know merely that the animal has been found at Woods Hole and Newport and from waters of the Chesapeake. Considering that the animal is so very widely distributed in Arctic, Antarctic and northeast Atlantic

regions, as well as the Adriatic, it is probably common along many American coasts where as yet overlooked.

In seeking to refer the different forms of the bottle animalcule found thus far in American waters to known or new species we are confronted with ignorance of the anatomy and the life history of the animals and thrown back chiefly upon the secreted bottle or case, since it is this alone that is commonly preserved, and since this also presents preserved characters of form and proportions.

In the life history as known there has been no restriction of the possibilities of form and size change possible to a single individual. We know that there are free-swimming forms as seen by Claparède and more fully studied by Wright and confirmed by Andrews and by Penard.

In some cases these swarmers arise from fission of the parent as Möbius found to be true, but in many instances the free-swimmers that swarm out are only the old forms transformed into simpler larval shapes that have later to make new bottles and then become again complex in structure. In the former case the result of fission is one free-swimmer of small size and one remnant individual left to complete its perfect organization in the old bottle. There are thus large and half-sized forms: both perfect sedentary individuals and imperfect swimming larvæ. Moreover, we find that not only may each individual greatly change its shape from muscular contraction, but may change both shape and bulk under conditions other than the optimum of good feeding environments. Nothing is known of any conjugation and any influence this may have upon form and size.

While it is easy to assume that all the known forms of bottles may prove to be the products of but one and the same species widespread all through the various oceans of the world, evidence for this is lacking, and not having sufficient anatomical basis for classification, we must as a practical expedient adopt the plan of Carl Dons and determine the species by the form and size of the bot-

tles—ceding the point that these species may well have but a very temporary and artificial value.

That the species determined from characters of the temporary dwellings of the animal may, however, prove to be real species is indicated by the following considerations.

The bottle or case is a secretion from the surface of the animal, and, as seen by Wright in 1861, the bottom part, or sac, as we call it, is made first, and then the neck or tube added, and finally the lip at the mouth of the bottle. In making the sac the animal flattens its body and assumes the size and the form that the sac will have when it is poured out and hardened all round about the body in this shape, leaving only the blunt anterior end of the body free from secretion, so that the hardened sac comes to have a hole in the anterior end and is a bilaterally symmetrical product duplicating the form and proportions of the animal as if the latter had been cast in the sac as in a mold. The form of the sac is the form of the animal at that period of its life cycle. Subsequently the size, length, spiral ornamentation, if any, and the perfection of the funnel or lip of the tube are all representations of the habit of the animal in the consecutive phases of the manufacture of the tube. The angle that the neck of the bottle, or tube, makes with the body of the bottle, or sac, is fixed by the degree to which the animal contracts its anterior part to rear it up away from the surface of attachment and general plane of the sac; the diameter of the tube is that of the head end of the animal; its circular section is that of the head end; its length is that of the gradual elongation of the entire animal which carries the head end gradually ever farther away from the foot end till the maximum length is attained; the spiral character of the tube, when present, is determined by the rotation of the head end and by the localized and radially differentiated selective secretions and contractions of parts of the head end; the final lip of the tube is added by special change of shape of the head end which

assumes a mushroom form and secretes from its under surface. At any one period of making of tube the tube expresses the resultant of the two components, contraction and secretion by the body at that moment.

While the entire animal never has the shape of the tube, yet each part of the tube exactly fits the head end of the animal as it progresses away from the foot in coordination with the elongation of the whole animal. The completed structure represents a solidification of the form rhythms of the animal as does the shell of a gasteropod or the successive exoskeletons of a lobster or the hard envelope of a rhizopod, and differs from most of these chiefly in that the animal only temporarily assumes the forms expressed by the dwelling, and later lives freely movable in the dwelling and capable of leaving it by simply detaching the foot-end from the bottom of the sac.

How precisely the bottle represents the animal was seen in one instance when camera drawings of two successive bottles made by the same animal exactly coincided. If, then, the different shapes and sizes of bottles do not mean different species, it is because the different activities and forms of these animals are not specific, but only varietal or individual differences, or differences due to changing conditions, such as food, or to different successive internal states connected with internal rhythms. In ignorance of the possible changes of form any animal may go through, we may, for practical purposes, follow Carl Dons in describing the bottles as expressions of forms that *may* be specific in value.

Of the anatomical characters in *Folliculina* that may be made use of in classification, the nucleus has been emphasized by Dons, who would regard a moniliform nucleus as the attribute of one group of folliculinas as represented by Mueller's original species, while all others have a single lobed simple nucleus. But this is of no avail, for in the first place, the folliculina thoroughly studied by Möbius had moniliform nucleus but its dwelling can not at all be confounded with that of Mueller's

Folliculina, and Ryder expressly mentions the long-beaded nucleus in the form *Freia producta*, which is most remote from Mueller's simple form; and, in the second place, I find that the commoner Chesapeake *Folliculina* has a moniliform nucleus and is by no means close to the *Folliculina* of Mueller.

Thus the moniliform nucleus is not restricted to animals in sacs of the Mueller type. Moreover, observation shows me that in the commoner Chesapeake *Folliculina* the nucleus may pass from the moniliform shape to more and more simple shapes, resembling the elliptical nucleus of so many other species; that is, just as was to be expected from the findings of Johnson in *Stentor*, the form of the nucleus is not constant, but a very long moniliform nucleus may fuse into a short elliptical shape. The same change was observed by Sahrlage.

Whether the nucleus is condensed or nodulated can then be but a poor basis for classification of the Folliculinas.

Till recently no micronuclei have been described in Folliculinas, though known in *Stentor*, and Carl Dons has used this as basis for separating the Folliculinas from the *Stentor* family; however, in two forms of Chesapeake Folliculinas I find minute darkly staining bodies associated with the macronucleus which may well be micronuclei, though their function has not been observed.

The only other anatomical character available seems to be the form of the anterior part of the body which is in some Folliculinas a funnel, and in others a funnel with two sides, more produced so that they may even form long arms likened by Ryder to obstetrical forceps, and again, in another species, by Wright, to the long ears of a hare.

But here again I find that an animal may have in its periods of maximum expansion and feeding activities exceedingly long arms, which in retracted states during adverse conditions may be very greatly reduced and modified in form and proportions, and there are also transi-

tion stages constantly found in which the arms are either being regenerated, or "rediffer entiated," or reduced, to vanishing point.

It is precisely the great development of the edges of the funnel that makes the folliculina an advance upon the simpler state found in the stentors, so that the more simple folliculinas are those with a peristome readily referable to the stentor state, while the very highly differentiated folliculinas with extremely long ligulate lobes right and left from the edge of the funnel are the most remote from the stentors.

While in classification the relative amount of development of these lobes is evidently of great importance, it will require observation of many living specimens, as a rule, to determine whether a given specimen has short lobes from its present stage of development in the individual life cycle or from its permanent place in the stage of evolution from the stentor-like ancestor. On the other hand, the presence of long ligulate lobes will at once determine a high stage of individual and phyletic advance and place the specimen in the highest group of anatomical development.

But until the possibilities of change of form in each individual are known, and in the probable possession of only poorly preserved specimens, the practical expedient will be to adopt much of the procedure of Dons in making use of the forms of the dwellings in the description of what may for the present be regarded as species within the group of Folliculinas.

Relying, then, largely upon the bottles as indicative of specific differences, in the American Folliculinas, so far known, we may tentatively adopt the general subdivisions of Carl Dons, retaining the genus *Folliculina* for the small, very wide sacs with short simple tubes.

No form of this restricted genus has thus far been reported from the American coasts. Whether such forms are anything more than starved, depauperate or imperfectly developed Folliculinas may well be doubted.

The genus *Semifolliculina* he has invented for bottles of narrower form with longer necks, often spirally ornamented and provided with a collar or lip. He says that one of the most widely distributed, *Semifolliculina boecki*, occurs in North America, and probably he had in mind the specimens described by Leidy as *Freia americana* as above related. However, the description given by Leidy speaks of the "convolvulus-like mouth" of the tube, which is plainly shown in an unpublished sketch (Fig. 414, Vol. V, of Leidy's MS. drawings) made by Leidy, which, with his notes, were kindly copied for me by Professor J. Percy Moore, who succeeded in finding it among unpublished material left by Leidy. This sketch also shows not only transverse lines on the tube, but longitudinal lines that may be compared to those of Dons's *Semifolliculina gigantea* (though Leidy may have drawn some of the lines to bring out curvature). At all events the very wide lip of the tube recalls the lip of *S. gigantea.* The size of the animal stated in Leidy's published notice is almost a fifth of a line. In his manuscript certain measurements would on this basis mean that: length of animal is 416 μ expanded, but 298 contracted; length of entire bottle 416 μ; width of animal 166 μ, but at narrow neck below terminal funnel 83; width of expanded funnel and lobes, that separate "like a labiate flower," is 139 μ. From the sketch the evident nucleus is a rounded mass that might be 40 μ in diameter.

Dons has given measurements of various parts of bottles of *Semifolliculina boecki* in contrast with those of *S. gigantea* from which it appears that the animal seen by Leidy was rather larger than the tubes of *S. boecki;* thus the combined lengths of sac and tube in *S. boecki* are 265–410 and for *S. gigantea* are 250–1000; the width of sac, 105–135 in the former and 230-300 in the latter. Leidy's animal was apparently 166 wide and its bottle 416 long. Thus both width of lips of tube and dimensions as far as known tend to place Leidy's animal in *S. gigantea* rather than in *S. boecki.* However, the fig-

ure and the description of "vase-like tube" emphasizes the short neck of the bottle and makes the reference to *S. gigantea* doubtful.

A second *Semifolliculina* is known on the statement of Dons to have been found in material from the eastern coast of America, and this is his *S. planorbis*, characterized by very narrow tube and wide sac. This is suggestive of some of the apparently depauperate or dwarf forms occasionally met with in the Chesapeake and may prove to be but a transitory condition due to conditions of food or other factors and not a permanent form.

The folliculina found at Woods Hole, Mass., has not been described. In 1914 Dr. Elmer J. Lund observed these Folliculinas on stems of *Campanularia, Eudendrium* and *Bugula* from the wharf of the Bureau of Fisheries and in letters to me sketched the sac and tube with expanded lips and absence of spiral. Apparently the form and proportions are much as in the sketch of Leidy, but with longer tube. Professor Lund observed the free-swimming forms several times during the summer in various cultures and saw the formation of new bottles in June.

Some preparations of *Obelia* mounted many years since, probably at Woods Hole, Mass., about 1888, and very likely by Professor Brooks, have yielded me several specimens of *Folliculina* that are evidently the same forms as those seen by Lund and probably the same as those of Leidy. The animals are exceptionally well preserved, in dwellings that give the following measurements:

1. One sac attached its whole length to *Obelia* is 175 μ long and 50 μ deep, while the tube arising from it nearly at right angles is only 50 μ above the top of the sac, is 37 μ wide and flares out at the lip to a width of 62 μ. The animal drawn into its sac is 162 μ long and 35 mm. in greatest diameter, with spheroidal macronucleus 15 μ in diameter. The ligulate lobes are 75 μ long and 12 wide and plainly show the characteristic adoral zone proceed-

ing from the vestibular spiral out to the tip of the left arm, thence back to the dorsal curve, out to tip of right arm, and then along some distance dorsal to the ventral edge of the right arm, to end abruptly at entrance to funnel.

2. Another specimen seen from above has 250 μ length of tube and sac combined (100 tube, 150 sac), width of sac 75 μ, width of tube 40 μ, width of flared lips 75 μ. The macronucleus is 20 by 15 μ and the oral lobes the same size as in preceding specimen; length of animal 250 μ, greatest width about 60 μ, since it was but partly contracted.

3. A third empty case has sac 125 μ along shorter dorsal side and tube 125 long. Depth of sac 65 and tube width 37, width of flaring lips 50 μ. The axis of the tube rises about 135 degrees away from the axis of sac.

4. Another empty sac has the same length, but depth of only 40 μ, and the tube was but just begun or else broken off, with diameter of about 35 μ.

5. Another specimen has sac 150 long with great width of 80 μ, short tube 88 long and 40 wide with lips 67; it was seated in the branching angle of the hydroid and faced toward base of hydroid.

The contained animal was much contracted into sac, 150 by 60, with nucleus elongated 15 by 25, with lobes 37 by 14 μ, and the left lobe terminated in a papilla $2\frac{1}{2}$ by 10 μ, recalling the *Freia stylifer* of Wright that was stated by Ryder to be probably but a variety and which represents a temporary state as we see it in Folliculinas in the Chesapeake Bay.

6. The sixth specimen measured: sac 125 by 80, tube 150 by 40 with lips 55; inhabited by animal remarkably well expanded, having main body 166 by 40 with nucleus 17 by 22 and lobes stretched out to 92 μ and 10 to 12 wide. This animal contained a large diatom as food, while some of the others contained masses of detritus as if bacteria in digestion. Within the sac next the animal are several nucleated masses, either foreign protozoa or possibly

fragments of disintegrated *Folliculina* arising from division and dying.

Comparing these measurements with those given by Dons for *Semifolliculina boecki,* we see that they are somewhat smaller except for the length of tube; but in the present state of ignorance of limits of species it would be folly to separate this from the *Semifolliculina boecki,* which includes the so similar *Lagotia viridis* of Wright, which Dons has separated from the simpler *Folliculina ampulla* of Mueller.

Not making a new species of the Woods Hole form, we may tentatively refer it to *Semifolliculina boecki* as exhibited in the above evidence; moreover, the form from the adjacent region of Newport described by Leidy, though much like some of Dons's smaller *S. gigantea,* may be the same as *S. boecki,* and we thus have probably this one species along the New England coast, together with the narrow-necked form *Folliculina spirorbis* as quoted by Dons.

Yet along with the above six specimens is one that is aberrant. Its sac and tube in one line stand out freely from the hydroid, attached only at the base. The tube enlarges where joining the sac and envelopes it as a swelling within which the edges of the sac end as free lip or inturned shelf, producing the appearance of a circular valve standing inward from the wall. This empty case is thus much like the *Folliculina telesto* of Laachmann as figured by Dons from Dröbak, page 88, Fig. 2, and later called *Parafolliculina violacea* by Dons.

The dimensions of this single empty case are: length, 250; greatest width, 63; length of tube, 82; width of tube beneath collar, 30; width of collar, or lips, 42; width of swollen tube where embracing mouth of sac, 51; width of sac just below this swelling of tube, 40; diagonal width of inner projecting flange that might be called a valve, 7½. Compared with Laachmann's specimens 200–260 by 60 and with Dons's 260–310 by 55–90, it closely resembles the former in size and the latter in appearance and pro-

portions, as seen in the above Fig. 2. This single specimen agrees very well in size with the six others on same material, but differs in the narrow lips and the swollen base of tube embracing the sac, as well as in the attachment of a sac by end only.

Knowing the normal mode of secretion of sac and of tube, one is tempted to suppose that a single exceptional specimen like this may have arisen by some fault in attachment of a swimmer followed by lack of proper rhythm of secretion, so that sac was attached by end and not along whole side, and that later the animal abnormally started to secrete a second collar within the old junction of sac and tube, bulging out the tube while still soft by pressure of its persisting mushroom and making an inner rim or shelf to represent an imperfect collar after it had already made an insufficient one at the mouth of the abnormally narrow tube. The whole structure would thus be an abnormal product resulting from slight abnormalities in secretional activities of the animal after unusual attitude in attachment. Such an hypothesis for explaining the telesto shape might be extended to all the telesto forms seen hitherto by Laachmann and by Dons, and these are significantly few; thus Laachmann found but *one* specimen on material from Sumatra and Dons found 20 after much search on Eudendrium from the Adriatic and but about a dozen amidst very many *F. ampulla* (*i.e.*, *S. boecki*) from Dröbak, and none at all from North Norway. A form occurring but rarely and found in Sumatra, West Australia, Norway and Woods Hole, Mass., may well prove to be but an abnormality rather than a real species.

The same suspicion of abnormality attaches to four additional sacs found empty and clustered together on the above hydroid material from Woods Hole. Apparently unfinished, they are characterized by great breadth and shortness and by narrow openings where the tube had not yet been added. With length of 110–113 μ, these sacs were 90 μ wide. They thus recall proportions of

Mueller's Folliculina, but the form is evidently so different in each of the four that they may be thought of as greatly shortened *Semifolliculina boecki* in which a premature change of axis in secreting has made the sac very deep where passing over into the tube, which is then expressed as part of the sac and left unfinished. The whole is like a short club foot with swollen ankle.

For the present all the known normal material from the coast of New England (except that referred to by Dons as *S. planorbis*) may be regarded as belonging to those forms described by the name *Semifolliculina boecki* and closely akin to the original *Folliculina ampulla* of most authors.

Turning now to the southern coast, the bottle animalcules first seen by Ryder in the Chesapeake were referred by him to *Freia producta* of Strethill Wright, September 3, 1880. This animal 1,000 μ long extended and 100 when contracted has a dwelling compared to a stocking with spiral ribbon of four to twenty-four turns to the right. Found in vast numbers on oyster shell with Bryozoa, its occurrence agrees with that later reported up the bay by the present author. What is evidently the same form has been seen by me in the Severn and other parts of the Chesapeake Bay in 1912–15 and described without specific identification.

In Dons's classification these Chesapeake forms are evidently *Semifolliculina* and might be included in the widely variant *S. boecki,* but that the tube is so much longer and the collar so relatively narrow.

Moreover, it has the gregarious habit described by Wright, the free-swimmers being stuck together in a secreted "colletoderm," and may be identified as the same as his *Freia* (*Lagotia*) *producta,* if we grant that in his sketch of the animal and bottle he overemphasized the "immensely prolonged" tube in proportion to the sac, which he figures as relatively too short for his comparison to a jack boot.

If, then, we may retain the name producta as signify-

ing a long spiral tube with narrow collar, we will separate it from the other four species *S. gigantea, S. planorbis, S. similis* and *S. boecki* recognized by Dons and may add the characteristic feature of the animal that its nucleus is generally moniliform and the lobes very long and ligulate so as to be compared to the ears of a hare in length by Wright and from their curvature of surface to blades of obstetrical forceps by Ryder—far removed from the funnel appearance of many of the smaller and earlier forms that had been described.

Tubes that have had second additions added to them, described by Wright, were also sometimes observed both by Ryder and by Andrews.

While the ordinary form of the Chesapeake is thus a much longer tube and markedly spiral as compared to the simple New England form, so that it may be provisionally regarded as specifically distinct and referred to *Semifolliculina producta,* there are also other forms of bottles occurring sparingly with the long tubes that are more simple and short than the New England forms, though they may have an added complexity regarded by Dons as a sort of valve and relegating them to the genus *Parafolliculina.*

Of the two species recognized by Dons the Chesapeake form is evidently *Parafolliculina amphora.* Characterized by the wide flat sac attached along its lower face to substratum and joined to short tube which swells out around mouth of sac and then rapidly diminishes to end with upward turn and narrow mouth with little or no collar. The whole enveloped for the most part in a halo of soft secretion and the junction of sac and tube characterized in many specimens by an internal valve-like set of membranes or modifications of the edges of the sac where jutting into the swollen tube. The animal is simple with single nucleus and short arms or funnel and nearly colorless.

The measurements given by Dons for specimens from Norway and Iceland are: length over all 110 to 150, of

which sac is 73, 82, 100, 112, when tube is 27, 27, 50, 37. Width 90–120, narrowest width 40–70. Diameter of swelling of tube 50–80, width of tube about 30–50. Diameter of nucleus from 15–32.

The Chesapeake forms have a much flattened sac, some 130–140 long by 195–110 wide, but only 30 deep. The exact point of passing to tube is various; the tube may be regarded as 25–55 wide and swelling to 57 wide, though it may be but 25 at actual mouth, which rarely has a collar, but may have a flaring of 6 μ. The length of tube may be 30, making the length over all 175. The tube may turn upward from attachment nearly 50 μ when sac is but 30 deep. The nucleus is from 15 by 17 to 15 by 27.

The halo of secretion about sac is some 7 μ or more in thickness. Characteristic of some specimens is the valve, so-called, relied upon by Dons as of generic value. This appears as a dorsal and ventral flap of membrane within the sac at its continuation as the tube and projects forward. These two membranes converge to meet below the center of the cavity, which they close off more or less completely. They vary in number and position and often seem to be lacking.

A description of these amphora forms of the Chesapeake will be published elsewhere and we will here merely add a summary of the foregoing consideration of the probable position of the known American folliculinas in the tentative scheme of classification of the family.

Following Dons we may separate the Folliculinas from the Stentors on account of the more or less marked development of the body as lateral lobes which make a funnel leading toward the mouth and which may be regarded as a specialization of the more primitive feeding apparatus found in *Stentor*.

The Folliculinidæ are thus Heterotrichia with spindle-shaped body prolonged as lateral lobes to form a funnel leading toward the mouth and marked ability to secrete dwellings composed of a sac-like part more or less prolonged as a tube which may reach great length and exhibit spiral structure.

The sac is fastened by secretion to some foreign body and the animal lives sedentary till such times as it may break loose from attachment of foot end to base of inside of sac and then swim free to soon secrete another sac and tube. In free-swimming individuals, lobes and mouth may be absent and later reconstructed. Contractile vacuole absent.

The nucleus is round, oval or moniliform. The micronuclei may be many and minute. Cross division results in halves, of which one may remain and the other escape from the dwelling; the size differs much in different individuals. Complete life cycle, when known, may show that some of the apparently specific forms are but stages in life cycle of others.

Species based so largely upon the forms of the secreted dwellings may eventually prove to be but results of diverse secretional activities within one species.

Conjugation unknown; reproduction by transverse fission follows nuclear condensation and dedifferentiation of peristome; the posterior half grows a new mouth and peristome and soon occupies the old dwelling, while the anterior half swims free with no mouth and simple spiral membranelle zone, secretes a new dwelling, and differentiates new mouth and peristomal apparatus.

The old genus *Folliculina* may be conveniently divided into tentative subdivisions as suggested by Dons; based chiefly upon shape and proportion of the secreted cases.

These groups may be spoken of as genera, namely, *Folliculina, Semifolliculina, Parafolliculina* and *Pseudofolliculina.*

In *Folliculina* Lamarck, as restricted by Dons, the sac is commonly as wide as long, the tube is short and without collar and there is no spiral nor valves.

The only species are the original *F. ampulla* of Mueller and the *F. paguri,* which was Giard's *Pebrilla paguri,* and seems to be an abnormality. The fresh-water *F. boltoni* seems to be the same as *F. ampulla,* and is recorded from England, Switzerland and from France (as the *Ascobius* of Henneguy).

In *Semifolliculina* the sac is longer and the tube may

be very long, with a collar and more or less spiral marking, but no valves.

Some six species may be recognized. The widespread *S. boecki,* being separated by Dons from the old *F. ampulla* and bearing the specific name of Claparede's *Cothurnia,* includes the *Freia ampulla* and *Freia aculeata* of Claparede (which are only stages of growth transformations), as well as the *Lagotia viridis, L. atropurpurea* and *L. hyalina* of Wright. Similar but much more evolved in specialization of tube is the *S. producta* of Wright.

S. gigantea of Dons is the same as Laachmann's antarctic *F. ampulla* and as Stein's *F. ampulla.*

The *S. elegans* of Claparede may belong here as tentative species with mouth of tube incised on one side, but this feature may well be accidental form.

S. spirorbis of Dons is well marked by absurdly narrow tube and is a very minute form that suggests depauperization.

S. similis recently described by Dons from south polar material possesses a very wide tube without spiral. The specimen figured shows tentacular projections of lobe such as we see in transformation stages.

Parafolliculina has a short tube which is swollen just above a narrow connection with the sac and may present internally membranes regarded as valves. There are two known species. The typical species is *P. amphora,* one of the smallest of the bottle animalcules found by Dons to remain the year through in some localities in Norway, while also known from Iceland and, we find, in the Chesapeake Bay. The other species, *P. violacea,* has the case attached only at the base of the sac and not along its entire ventral face as in all above-named species. If this feature is incident to some unusual behavior of the free-swimming stage when about to settle down and construct the sac, the two species may prove to be but one. It was found on the French coast by Giard and is known from south Norway, the Adriatic and West Australia.

Pseudofolliculina has no enlargement of the tube, but,

in fact, the tube and sac grade into one another and stand straight up from the attachment. This is by means of a long cylinder of cementing material, bringing the base of the animal well above the substratum. There may be a simple membraneous valve.

Of the two species, *P. mellita* was taken in the Antarctic in 1902–3 by Laachmann at depths of 350–385 meters and its case has a length .6–.7 mm., but this great length is partly due to the mode of attachment of the sac by means of a stalk of secretion. As this stalk is hollow and filled by a tenuous prolongation of the body of the animal, it may be that this genus also is founded upon individual idiosyncrasies of secretional activity.

The other species, *P. arctica,* has been formed by Dons to include the smaller but similar forms he found in Norway and finally separated from *P. mellita* as smaller, with narrower stalk apparently not perforated.

As the above eleven species have been most studied in England, Germany and Norway, it is natural that Folliculinas are known chiefly from those coasts; yet the known distribution of Folliculinas has already been extended to the Antarctic, the Mediterranean, the White Sea, as well as to the east and west coasts of the North Atlantic. On the east coast of the United States we seem to have four or five species in two of Dons's subdivisions of the old genus *Folliculina,* namely: the most specialized and best known *Semifolliculina producta* of the shores of the Chesapeake; the accompanying small and simpler form *Parafolliculina amphora;* the less well-marked form *Semifolliculina boecki,* first found at Newport, R. I., and later at Woods Hole, Mass.; and finally, as recorded by Dons, from material from the North American Atlantic coast the *Semifolliculina spirorbis;* and if Dons's *Parafolliculina violacea* be a real species, it also is to be credited to Woods Hole.

That some or all of the smaller and simpler forms arise from larger and more complex forms under changing conditions of nutrition in the successive phases of sedentary and free life is a tempting working hypothesis.

Whether these widely distributed marine protozoa which in some cases are able to live in fresh water may not be found in all parts of the world remains to be found out; with attention turned to their discovery, it may be hoped that knowledge of both the life histories and the taxonomy may soon be placed upon a firmer basis.

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SHORTER ARTICLES AND DISCUSSION

THE DUALITY OF EGG-SECRETION

THERE are two properties of egg-secretion which have attracted particular attention. One of these is the power of agglutinating spermatozoa (Lillie[1]); the other is the power of activating the egg (auto-parthenogenesis, Glaser[2]). These effects have been studied separately, but under normal conditions both must bear, in close affiliation, upon the events of fertilization.

I

We are still in the stage of hypothesis. We know that without exudates fertilization does not take place. This was first demonstrated by F. R. Lillie (*loc. cit.*), who removed the exudates. These, as I found later, can be rendered ineffective without removal, by the presence of charcoal.[3] In neither case is fertilization possible. We also know, from the investigations of Lillie, that the agglutinating agent can be neutralized in two ways: either by spermatozoa, or, by some derivative from the egg itself. Inasmuch as fertilization is prevented by this derivative, Lillie has called it "anti-fertilizin."

Lillie also discovered that the blood of the sea-urchin (*Arbacia*) has preventative effects, but these are different from those of "anti-fertilizin." There is in this case no interference with the agglutination reaction; yet the egg remains unactivated.

Miss Woodward[4] and I (*loc. cit.*) have extended the list of inhibitors to include such diverse things as the pigmented substances derived from testicular tissues; fatty and aqueous extracts of the eggs; and now, oleic acid and olive oil. However I shall consider further only the inhibitors discovered by Lillie.

II

These, together with the fact that the exudates are necessary in normal fertilization, are the foundations of the fertilizin

[1] *Science*, Vol. 38, pp. 524–528.

[2] *Biological Bulletin*, Vol. 26, pp. 387–409.

[3] The use of charcoal was suggested to me by Dr. G. H. A. Clowes.

[4] *Journal of Experimental Zoology*, Vol. 26, pp. 459–501.

hypothesis. According to this view, egg-secretion contains a body with two bonds; one normally unites with a sperm-borne valence; the other, with a valence borne by the egg. Agglutination is a symptom of the first union; activation of the egg, a symptom of the second. "Anti-fertilizin" occupies the agglutinating valence and hence the normal union of sperm and fertilizin is rendered impossible. The inhibitor in the blood, on the other hand, is effective because it binds the second valence of the fertilizin which in consequence cannot unite with its normal receptor in the egg. In the language of Ehrlich, fertilizin is an amboceptor with ovophile and spermophile side-chains; and, normal fertilization involves the formation of a chemical compound which, written in linear fashion, can be thought of as sperm-receptor—spermophile side-chain-ovophile side-chain—egg-receptor.

The keystone of the hypothesis is the amboceptor. It symbolyzes the effects of the secretion on spermatozoa and the effects on eggs; it symbolizes also the information gotten from the two inhibitors—the one that prevents agglutination and fertilization and the other which prevents merely fertilization. The amboceptor stands vaguely for the recognized duality of the secretion. The question is, can we precipitate the amboceptor from the realm of the symbolic and bring it within the sphere of the commoner conceptions of physics and chemistry?

III

First of all, are we compelled to think in terms of the amboceptor? The alternative, of course, is a two-body view. Lillie's book,[5] (p. 231) decides against this because there is "a parallel between absence or loss of agglutinating substance and the capacity of the egg for being activated. The same results would be attained if there were two substances concerned . . . but since the two effects . . . appear and disappear together . . . the writer assumed that they may be regarded as due to a single complex substance." Again (p. 232), in discussing the effects of the inhibitors, Lillie writes:

> This still does not prove that sperm agglutination and egg activation are due to the action of a single substance, but it shows again by a different method that the capacity for producing both effects is present

[5] "Problems of Fertilization," University of Chicago Press, 1919.

simultaneously in the egg secretion and the assumption of a single substance is the simplest hypothesis.

A much stronger argument for the one-body view can be advanced. "Anti-fertilizin," by hypothesis, prevents normal fertilization by occupying the spermophile side-chain; yet, "anti-fertilizin" also prevents auto-parthenogenesis (Woodward). This, we might suppose, would indicate a relationship between the two side-chains, such that removal from the reaction system of the one also results in the removal of the other. This state of affairs is actually realized under certain conditions. For example, I have been able to isolate from charcoal materials with agglutinating and activating properties. However, if we infer from these observations, a single body, the reaction of "anti-fertilizin" with fertilizin can hardly serve as evidence that the latter has two side-chains.

IV

In 1918, Miss Woodward[4] reported experiments that bear intimately on the problem. By saturating *Arbacia* secretion with $(NH_4)_2SO_4$ she secured a white flocculent precipitate which after purification by dialysis proved to have very intense agglutinative powers but no capacity for initiating the development of the egg. She called this precipitate, agglutinin.

An initiatory agent, also, was precipitated. $BaCl_2$ was added to the secretion and after removal of the sea-salts, the primary deposit was treated with $N/10$ HCl. As soon as the acid had been freed from $BaCl_2$, acetone was used in excess to bring down a second precipitate, heavy and flocculent. This, after purification with absolute alcohol and ether, dried as a white powder, soluble in both sea-water and distilled.

This second precipitate had marked parthenogenetic effects, but no power to agglutinate sperm. Miss Woodward called the substance, lipolysin, a name which, as I shall show elsewhere, is justified since the material accelerates the hydrolysis of fats.

V

What bearing have these precipitations on the issue? According to Lillie's book, p. 240, "separation under the conditions of chemical analysis may possibly denote a splitting of a single substance of the normal egg."

A. The Ammonium-Barium Precipitations

1. The agglutinin can be salted out with $(NH_4)_2SO_4$; the lipolysin can be brought down with $BaCl_2$. If we divide a given exudate into two portions, we can precipitate in one, first the agglutinin and later the lipolysin, whereas with the other fraction we can proceed in a manner exactly the reverse.

2. Precipitation in the two cases differs inasmuch as the effective concentration of the $BaCl_2$ is $N/7.5$; that of the $(NH_4)_2SO_4$ in the neighborhood of $5N$.

If precipitation in the two instances is uncomplicated by chemical unions between precipitate and the reagents used to produce them, the case is decisive for the two-body view since it is unlikely that a molecule can be split chemically, merely because one part has solubilities different from those of another part. However, the case is probably not so simple. Very possibly we are dealing with ammonium agglutinate and barium lipolysinate. If this is true, then all we can say is that the amboceptor, present by hypothesis at the outset, breaks down between the agglutinating and the activating valence no matter which of the two groups is bound first.

B. The Charcoal Method

Charcoal removes practically the entire organic reaction system. This can be recovered in its essential parts, by subsequently treating the charcoal with $N/10$ HCl. From the clear solution so gotten acetone throws down a voluminous precipitate in two well-marked stages: the first fraction, without agglutinating powers, is lipolytic; the second strongly agglutinates the sperm.[6]

Very likely factors are involved in precipitation by charcoal which are not present when $(NH_4)_2SO_4$ and $BaCl_2$ are used. Very possibly the amboceptor is split by the charcoal; or, it may not be split until the acetone in the HCl reaches a certain concentration. In any case the cleavage of the molecule gives results identical with those gotten by Miss Woodward's methods.

C. Reactions to Heat

Lillie has shown that the agglutinating material is extremely resistant to heat. Exudate which has been boiled agglutinates spermatozoa perfectly well. It has, however, lost its capacity as a parthenogenetic agent (Woodward).

[6] It is very important to guard against impurities in the charcoal. These after extraction with HCl give a voluminous precipitate with acetone.

On the one-body view we must reckon with the following possibilities: (1) a rupture of the molecule; (2) the destruction or alteration of the ovophile side-chain.

VI

Looked at conservatively, the methods of precipitation alone do not enable us to decide the one-body–two-body issue. The same thing may be said of the results of boiling. One conclusion, however, is certainly warranted: there is a constitutional weakness in the amboceptor so pronounced that this molecule breaks down with the greatest ease and, under very diverse conditions, always cleaves in a manner that separates the spermophile from the ovophile side-chain. Indeed one doubts whether the amboceptor can hold its ovophile side-chain after the agglutinating group has united with the receptors of the sperm. This, if true, would be awkward for the theory.

VII

D. FILTERABILITY

Lillie has shown that exudates which have passed through Berkefeld filters no longer agglutinate spermatozoa. The agglutinating material, in this case, can be recovered, as Miss Sampson found last summer, by washing the filter-cone in sea-water. In the original filtrate I was able to demonstrate lipolysin.

It is conceivable that the agglutinating material is not filterable because of "chemical adsorption." If this is true, then filtration becomes merely another method for the chemical decomposition of the amboceptor. Yet, we can account in this manner for only a portion of the agglutinin held back. It seems very unlikely that the fraction which can be recovered by merely washing the filter-cone, was held chemically bound. Moreover, on account of the metal band which holds the filter-cone in position, a remnant of the secretion invariably fails to pass through. This remnant has a higher agglutinating value than the original exudate. It seems safe to conclude that the agglutinating material is held back mechanically. If correct, these considerations based on filtration are conclusive, for we know of no cases in which a substance is chemically decomposed merely because the whole molecule is unable to get through the pores of a filter.

The results of filtration seem to me to necessitate the two-

body view. None of the facts explicable by the amboceptor appear to become inexplicable when this body is analyzed into an agglutinin and a lipolysin. As for the difficulties of constant association, these are no greater in this case than the difficulties which arise because serum albumin and serum globulin always occur together in the blood.

OTTO GLASER.

AMHERST COLLEGE,
February 7, 1921

DESCRIPTION OF A PECULIAR YOLK MASS IN THE OVIDUCT OF A HEN

A DESCRIPTION of this specimen seems desirable for two chief reasons: First, because of its unique nature; second, because it supplies the data with which to answer the question whether reverse movement, possibly antiperistalsis, occurs in the formation of double eggs and similar anomalies.

The specimen was presented to the histological laboratory by Ashton Barbour, of Charlottesville, Va., six hours after it had been removed from an apparently normal year-old hen. He described it as having been taken from the "egg-bag." When questioned, he was positive that he had noticed a number of developing eggs, "little yellow balls," attached to the dorsal surface of the abdominal cavity. His anatomical observations stopped at this point.

The specimen was roughly egg-shaped, and of a yolk or yellowish-orange color. Between ends it measured 9½ cm. Its diameter at the point of greatest width (about one third the distance from the wider end) was 8 cm. When opened the mass was found to contain an egg of average size, with a shell of normal hardness and thickness. The egg was not exactly in the center, but was placed slightly to one side and towards the larger end, causing a variation in the thickness of the lateral walls of the enveloping mass (Fig. 1). At the thickest point the lateral wall was 2 cm. thick, at the thinnest point 1 cm. At the larger end the wall measured ¾ cm. in thickness, at the smaller end it measured 1½ cm. in thickness. The weight of the enveloping portion of this yolk mass, after the enclosed egg had been removed, was about 190 gms. The general ovoid shape of the mass was presumably determined by the enclosed egg.

The mass was made up of layers of yellow, yolk-like material

between which were scattered irregular laminæ of a glairy, mucus-like substance (Fig. 2). In places these laminæ had apparently hardened to form clear, firm, gelatinous areas. The outermost yellow layer was about 1½ mm. thick, and completely encircled the mass.

On one side, at the point of greatest diameter, there was a

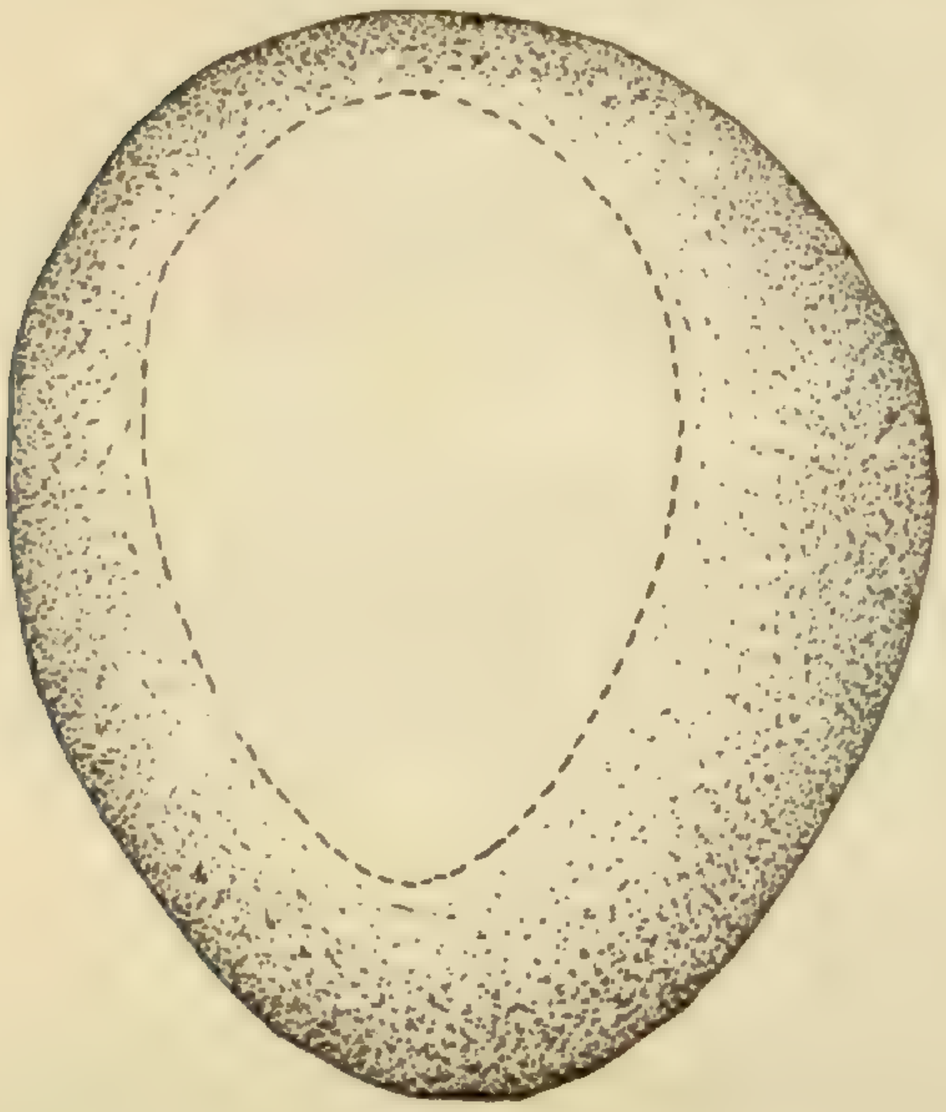

FIG. 1.

FIG. 2.

FIG. 1. Diagram of yolk mass from oviduct of hen. The inner broken line represents the outline of the inclosed normal egg. The peripheral stippled area represents the laminated envelope of yolk and albumen. Natural size.

FIG. 2. Diagram of transverse section of the yolk mass. The central circular area represents the inclosed normal egg. The peripheral stippled lamellæ represent layers of yolk, the clear lamellæ, layers of albumen. Natural size.

shallow depression, about 2 mm. deep, almost the size of a dime in circumference. This was due to a thinning of the two external enveloping layers at that point. A dark, reddish discoloration partly surrounded this depression in the form of a crescent. There were a number of small granule-like hillocks, about the size of a pinhead, on the surface of the smaller end. These elevations probably represent casts of the mouths of the oviducal glands, produced under pressure of the enlarging mass against the constricted confines of the oviducal walls.

When the egg which the mass inclosed was removed and opened, it was found to be filled with a yellowish liquid, in which there were bits of a translucent and whitish mucus-like substance, the remains most probably of the disintegrated chalazæ. The odor of this liquid was not offensive. It may be best described as musty.

Portions of the yolk mass were imbedded in celloidin, sec-

tioned, and the sections stained with hematoxylin and eosin. Transverse sections through this laminated cortical material revealed layers of yolk granules and spherules intermingled with layers of clear, hardened, egg-white. There were no indications of the presence of shell, or any unequivocal evidence of shell membrane, in any of the sections.

Abnormal eggs have been observed and discussed by biologists for many years. In the AMERICAN NATURALIST for January, 1906, G. H. Parker (4) has treated the subject of double hens'-eggs, "ovum in ovo," very fully. He reviews much of the previous literature on the subject and describes several specimens of his own, similar to a specimen of a large, double egg which belongs to the laboratory of histology here. Parker supports the theory of Davaine (2) and others concerning the formation of double eggs.

Briefly the theory is this: The egg is moved by peristalsis from the ovary to the distal end of the oviduct. As it passes down the oviduct it receives the usual coverings of albumen, shell membrane, and shell. The egg is now a normal egg, ready to be laid. But for some reason, instead of the egg being laid normally, antiperistalsis occurs and the egg is carried back up the oviduct. In the upper portion of the duct it meets another developing egg coming down. The two pass down together. Albumen is laid on and a common shell covers the whole mass. We now have a giant egg, approximating the size of an ostrich egg, which contains a second complete normal egg along with its own yolk and albumen.

Curtis (1) has described a number of interesting anomalies in hens' eggs, including double eggs and other anomalous specimens, either with a membrane only or with both shell and membrane. She reports finding eggs in the body cavity of fowls whose oviduct had been ligated in the isthmus, or shell gland. She does not venture to commit herself, however, as to whether antiperistalsis is the means by which the egg is carried back up the duct. Patterson (5) describes a specimen which has two shell membranes. He explains this condition on the assumption that antiperistalsis had occurred twice before the egg was laid. Hargitt (3) describes an interesting gourd-shaped egg. None of these authors, however, mention an anomaly similar to our specimen.

What may be assumed to have happened in the formation of

our specimen was this: The first egg which left the ovary of the young hen passed down the oviduct normally and had albumen and shell laid on in the usual manner. The egg passed on into the lower part of the uterus (shell gland), but, due to injury, congenital occlusion of the vagina, or some obstruction, the egg could not be laid. Such interference with normal oviposition, either congenital or acquired, stimulated a reversed movement (probably antiperistalsis) and the normal egg was carried back up into the oviduct and lodged there. More eggs left the ovary, took on albumen as they passed down the duct, but coming into contact with the preceding egg which occluded the duct, where broken by pressure, and the soft yolk and albumen collected about the obstructing egg. In this way the yolk mass about the egg acquired its large dimensions.

We can thus locate very closely the exact position of the anomalous yolk mass in the oviduct of the hen. Since it contained layers of albumen it must have lodged below, or in the lower part of the portion of the oviduct where albumen is laid onto the yolk; and since there was no shell whatever within the cortex of the mass, it must have lodged above the point in the oviduct where shell is formed. Again, antiperistalsis, or at least reversal of normal movement, must have occurred because the inclosed egg comprised a shell, and so must itself have gone the full length of the oviduct into the uterus, while at the time the enveloping yolk mass was formed the original egg must have been above the shell-forming level.

The above evidence, combined with the evidence of Davaine (cited by Parker) and Curtis, who report finding soft-shelled eggs in the body cavity of fowls, seems to prove conclusively that something of the nature of antiperistalsis in the oviduct does occur. The inference seems warranted with regard to our specimen, that if the included normal egg could have retraced its course down the oviduct in company with the next following egg, instead of lodging permanently in the preuterine portion of the oviduct, a common shell would have been laid onto the two eggs. This shell would have included the two together, and the result would have been an ordinary "ovum in ovo," similar to the ones described by Davaine, Parker, Patterson, Hargitt, Curtis and many others.

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ROBERT BATTAILE HIDEN

THE DEPARTMENT OF HISTOLOGY
AND EMBRYOLOGY,
UNIVERSITY OF VIRGINIA

THE HEREDITY OF ORANGE EYE COLOR IN DROSOPHILA MELANOGASTER[1]

THERE are three points of special interest in the heredity of orange eye color. First, the eye color is due to the presence of two sex-linked genes; second, these two genes may separate in the F_1 female when orange is crossed to the wild stock, producing in F_2, in addition to orange and wild type males, a third eye color called salmon[2]; third, when an orange male is crossed to the parent stock, reduced, only orange and wild type males appear in F_2.

Orange first appeared in the sixth generation of the plus selected line of the mutant strain reduced. Eleven males appeared from a single pair of parents. Several of these males (orange reduced) were mated to wild type females. All F_1 flies had red eyes. Twenty-seven F_1 pairs were mated (Table I). Of the F_2 males 850 were wild type (red-eyed), 785 were orange reduced, 585 were reduced, 586 salmon, 15 orange, and 6 salmon reduced. This behavior led us to suspect that orange

[1] Contribution No. 182.

[2] Since this paper went to press Professor Morgan kindly sent us some stock of garnet. The crosses show salmon and garnet to be the same.

TABLE I

F_1 ♀ WILD TYPE × F_1 ♂ WILD TYPE (FROM ORANGE REDUCED ♂ × WILD TYPE ♀)

Bottle Number	Female	Male					
	Wild Type	Wild Type	Reduced	Orange Reduced	Salmon	Orange	Salmon
789	194	65	42	61	46		
798	127	31	22	33	34		
804	92	15	19	30	11		
808	109	26	24	29	30	2	
814	72	17	9	14	13		
815	140	33	23	30	16	2	
816	123	22	16	25	14		
831	65	17	8	12	7		
854	77	30	11	14	9		
859	69	18	11	13	15	1	
860	66	18	13	22	13		
1,431	100	30	13	25	17	1	
1,372	174	48	39	31	31		
1,494	28	12	7	8	5		
1,495	173	45	34	37	30	1	
1,557	149	31	25	42	22	1	
1,569	112	87	25	29	19	2	2
1,570	106	20	20	17	17	1	
1,571	66	22	9	10	15		
1,674	111	26	27	27	33		
1,751	175	62	41	55	42	1	1
1,736	207	64	27	72	26		
1,724	174	55	21	36	24	1	
1,720	158	35	30	40	34	1	
1,441	111	39	27	32	21		1
2,431	74	22	20	22	19	1	
2,433	81	20	22	19	23		2
Totals	3,133	850	585	785	586	15	6

TABLE II

$F_1 \times F_1$ [FROM ♂ SALMON × ♀ WILD TYPE (FROM STOCK)]

Bottle Number	Female	Male	
	Wild Type	Wild Type	Salmon
1,612	146	65	54
1,665	123	51	34
1,668	175	69	68
1,679	121	69	54
1,682	130	68	54
1,689	161	63	56
1,701	117	54	62
Totals	963	439	382

eye color was due to the presence of two sex-linked genes, one of which by itself produced no visible effect, but when the two were brought together orange was the result. With this hypothesis in mind we attempted to prove or disprove it and believe we have demonstrated that the suggested explanation is the correct one.

First, some of the F_2 salmon males were mated to wild and the eye color shown to be due to a single sex-linked gene. An examination of the F_2 males when an orange reduced male is mated to a wild female (Table I), shows that practically all salmon males (586 out of 592) are wild type with respect to bristle number, and that practically all orange males (585 out of 600) are reduced. These results showed that the modifying gene which changes salmon into orange is closely linked to reduced. Most of the F_2 reduced red-eyed males then should carry this modifier and we should be able to produce orange flies in the second generation by mating these reduced F_2 males to salmon females. Such crosses have been made and the results show that the F_2 reduced males do carry such a gene which when added to salmon produces orange. The two genes for salmon and salmon modifier are brought together in the same chromosome by crossing over in the F_1 female (Table III).

TABLE III

(*a*) REDUCED ♂ (F_2 FROM ORANGE REDUCED ♂ × WILD ♀) × SALMON ♀

Wild type ♀ ..... Salmon ♂
966 823

(*b*) F_1♀ [FROM (*a*)] × ORANGE ♂ FROM STOCK

Bottle Number	Female			Male		
	Wild Type	Salmon	Orange	Wild Type	Salmon	Orange
260.....	233	66	134	248	117	116
261.....	102	49	62	85	44	47
262.....	157	52	94	184	74	61
263.....	69	23	38	63	42	29
266.....	45	20	34	54	23	24
Totals ..	606	210	362	634	300	277

That the modifying gene lies in the X chromosome rather than in one of the autosomes is clearly shown by the F_2 ratios when orange is mated to a wild female. If the gene were reces-

TABLE IV

F_1 ♀ WILD TYPE × F_1 ♂ REDUCED (FROM SALMON ♂ × REDUCED ♀)

Bottle Number	Female		Male					
	Wild Type	Reduced	Wild Type	Reduced	Salmon	Salmon Reduced	Orange Reduced	Orange
8,347....	133	112	60	76	72	4	43	1
8,348....	124	119	46	57	67		40	3
8,354....	88	105	47	43	80	4	47	
Totals ..	345	336	153	176	219	8	130	4

sive and in an autosome all the F_2 females should be wild type. Such is the case. Of the F_2 males, four should be red-eyed, three salmon, and one orange. If the gene were dominant, the ratios among the males should be four red, one salmon, and three orange. The actual results are given in Table I. We find neither of the former ratios, but 1435 red-eyed, 800 orange, and 592 salmon. The fact that there is a linkage between the genes for salmon modifier and reduced is further evidence that the gene for salmon modifier is in the X chromosome.

Orange eye color, then, is due to two sex-linked genes, one of which when alone produces salmon; the other called salmon modifier, when alone produces no visible effect, but when added to salmon produces orange. The discovery of such a modifying gene is not new as Bridges has demonstrated seven of them which modify eosin eye color. It does lend support, however, to the presence and behavior of such genes.

These same orange reduced males when mated to reduced females give a very different result from the cross to the wild. When crossed to reduced, orange behaves as a single sex-linked character (Table II). Why this difference? At first it was our impression that the reduced strain carried a non-crossover factor. This was disproved, however, by mating reduced to other sex-linked characters. The other alternative and the correct interpretation we think, is that the reduced strain is homozygous for the gene for salmon modifier. This is easily demonstrated by mating salmon to the reduced line. If the reduced line carries the gene for salmon modifier, then a cross of this line to salmon should give some orange males in F_2. Such is the case (Table IV). The reduced strain, then, carried the gene for salmon modifier before the gene for salmon appeared.

No attempt has been made to locate accurately the genes for

reduced, salmon, and salmon modifier. Sufficient crosses have been made, however, to approximate their positions, which are as follows: reduced, 5.24; salmon modifier, 5.94; salmon, 41.33.

Description of Tables

Table I gives the results of mating the F_1 flies produced by crossing an orange reduced male to a wild type female. All the F_2 females are wild type. Of the F_2 males 850 are wild type. 585 reduced, 785 orange reduced, 586 salmon, 15 orange, and 6 salmon reduced.

Table II gives the F_2 results of mating a salmon male to a wild type female. Of the males 439 were wild type and 382 salmon.

Table III (*a*) and (*b*), gives (*a*) the results of crossing an F_2 reduced male (from an orange reduced male $\times$ a wild female) to a salmon female. The females are wild type and the males salmon. In (*b*) the F_1 wild type females were mated to orange males. The orange males were used instead of the salmon to bring out orange in the females. Orange males and females appear. Hence the F_2 reduced males must have carried the gene for salmon modifier.

Table IV gives the F_2 results of crossing a salmon male to a reduced female. Orange males appear. Hence the reduced line must be homozygous for salmon modifier.

Fernandus Payne,
Martha Denny.

Zoological Laboratory,
Indiana University

AN APPARATUS FOR MICRODISSECTION

To secure the greatest accuracy in the control of needles for cell dissection an apparatus has been devised on the following plan. The needle is attached to a right angled triangular plate (Fig. *A*), each corner of which is moved by a milled headed screw. The working of any screw causes the movement of the plate about a line through the points of the other two screws as an axis, thus producing motion of the needle point in the three planes of space by manipulation of the screw heads. The nature of the bearings of the screw points on the plate eliminates all side play. The conical end of one screw works into a circular

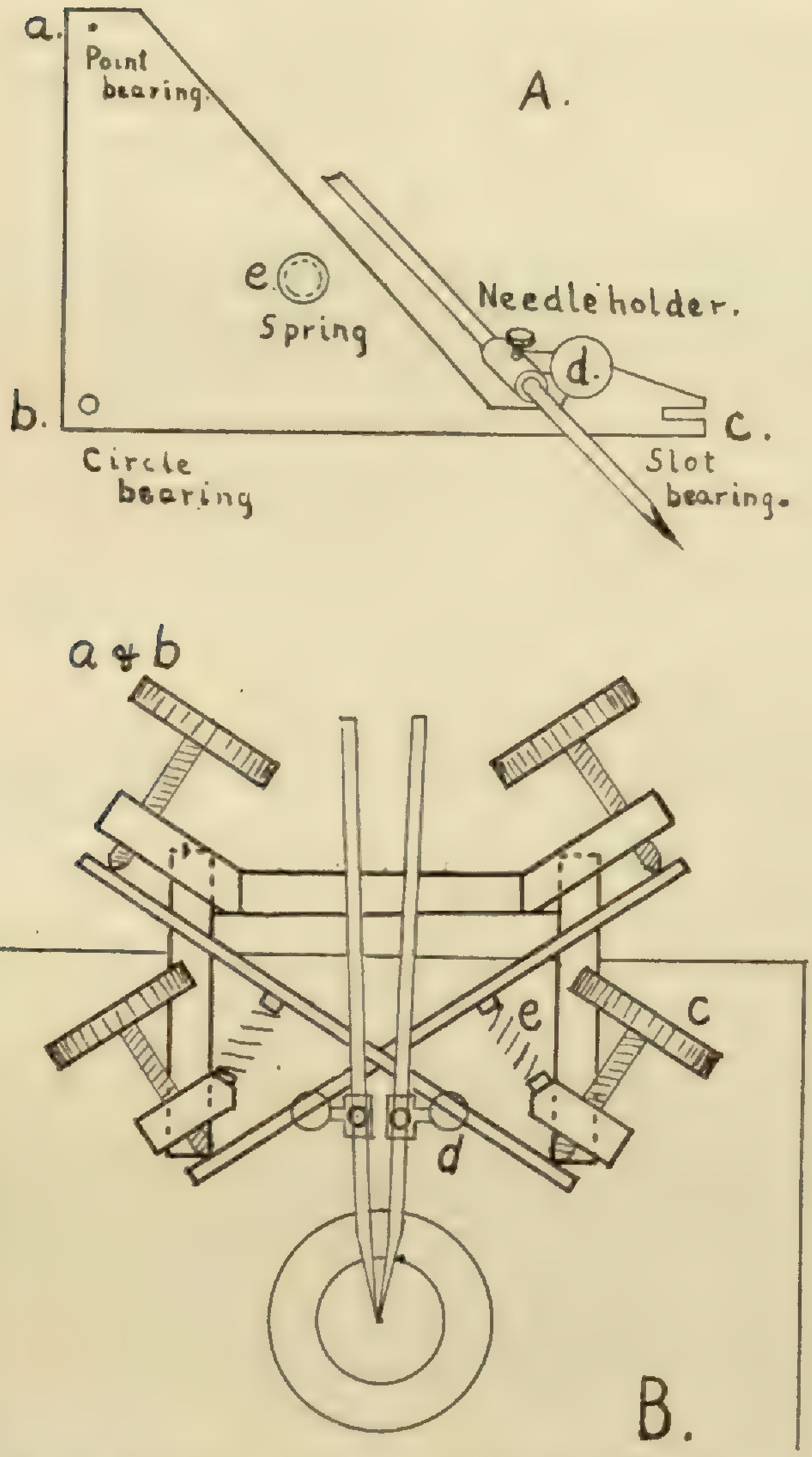

FIG. A. Detail of movable plate which bears the needle, illustrating the mechanical principles involved. See text.

FIG. *B*. Diagram of one type of the apparatus clamped to the microscope stage, designed to secure firm needle support close to the microscope focus. The fifth and sixth operating screws are directly beneath two of those figured (*e.g.*, *b* beneath *a*). The vertical movement and one horizontal movement of the needle point are secured by turning one screw each (*a* and *c* respectively); the second horizontal movement, by turning two adjacent screws together (*a* and *b*, with two fingers of one hand). Coarse adjustments are made by moving the needle at the ball-and-socket joint *d*.

hole through a corner of the plate (*b*, Fig. *A*); the conical point of the second screw works into a slit in its corresponding corner (*c*), the third screw point (at *a*) bears on the plane surface of the plate; these constitute what may be termed a cone-slot-point support. The cone-circle bearing prevents side slipping of the plate in any direction; the cone-slot bearing prevents revolution of the plate about the cone-circle bearing. A single spring (*e*)

against the center of the plate holds its bearings firmly against the three screw points, and takes up all lost motion perpendicular to the plate, in the screw threads.

This mechanism has been employed in two styles of dissection apparatus. In one type (Fig. *B*) the aim has been to secure a short needle length, and close proximity of the needle's support to the axis of the microscope lens, with consequent freedom from vibration of the needle and a long leverage in the control of its point. In the other type (Fig. *C*) the apparatus is set away from the microscope axis, to allow the greatest possible freedom of manipulation of objects on the microscope stage. In the former type, the plates bearing the needles cross at right angles, under the microscope nosepiece; in the latter type, they are set beyond the edge of the microscope stage, nearly parallel. Each instrument bears two pipettes or needles operated by three screws apiece. The needles may be attached to the plates either by wax, which when warmed makes possible a coarse adjustment of the needles, or by a universal ball and socket joint, clamped loosely enough to allow gross manipulation under the low power.

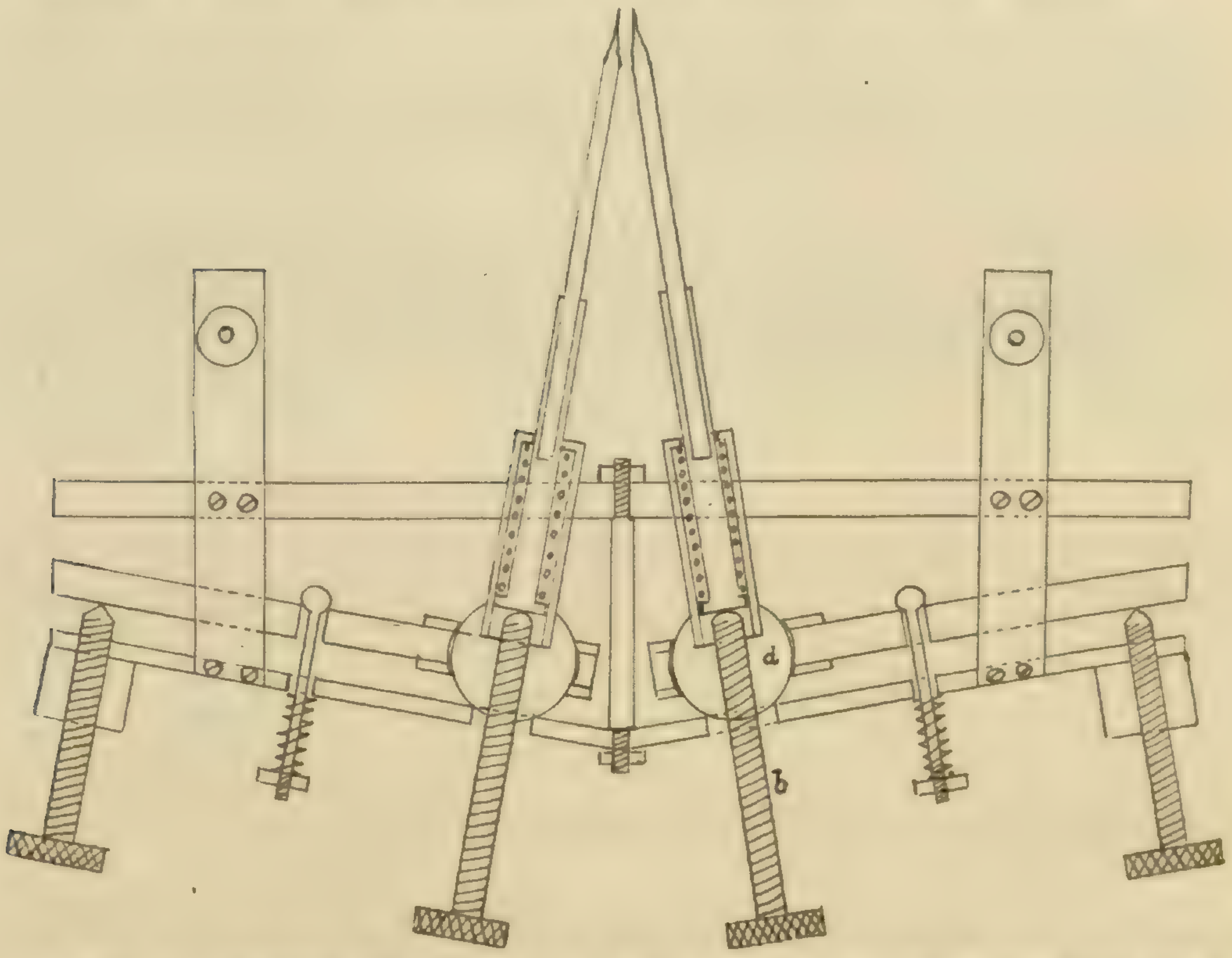

FIG. *C*. Diagram of second type of the apparatus, with parts arranged to leave the microscope stage free for more convenient manipulation of objects upon it. The ball-and-socket joint for coarse adjustment (at *d*) is here made to serve as a pivot in place of the corresponding screw point (*b*); this screw then works through this joint to produce one motion of the needle, thus avoiding the necessity of turning two screws together to secure this movement, as in Fig. *B*.

The advantage of this type of needle control over the sliding motions of the Barber pipette holder lies in greater ease and convenience of manipulation as well as in greater refinement of needle control. The simplicity of the apparatus is such that its manipulation requires no great degree of technical skill, and in its construction have been avoided as far as possible all elaborate and complicated adjustments. The screw heads turn easily enough to be rolled under pressure of one finger each. The complete apparatus for holding two needles may be made to occupy a three-inch cube, and clamped close under the nosepiece; five of its six screws can be operated with one hand, leaving to the other, the mechanical stage ratchets and the sixth needle screw adjacent to these. The needles may be held so close to their points that no vibration is noticeable, especially since no moving part is handled during manipulation. The needle points move in arcs of circles, but since the ratio of length of arc to radius is small, these are virtually straight lines, in the plane of, and perpendicular to the plane of the focus of the objective.

A more detailed account of the working of this instrument, and a description of various devices that may be added to facilitate needle manipulation, will be included in a subsequent paper dealing with the results of its application to the study of protoplasmic activity.

GEO. H. BISHOP,
C. E. THARALDSEN

ZOOLOGICAL LABORATORIES,
NORTHWESTERN UNIVERSITY

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THE RELATION BETWEEN BODY SIZE AND ORGAN SIZE IN PLANTS[1]

DR. EDMUND W. SINNOTT

CONNECTICUT AGRICULTURAL COLLEGE

IN the animal kingdom, particularly among its more highly specialized members where the primitive condition of indefinite multiplication of similar organs has given way to a high degree of differentiation, there is necessarily a close correlation between the size of a given organ and the size of the organism of which it forms a part. A particularly large individual will tend to have proportionally large bodily structures, and *vice versa*. In the case of the higher plants, however, with their multiplication of similar organs and their notably lower degree of organization and individuation, an interdependence between body size and organ size is certainly much less obvious. We need only to call to mind the general similarity between leaves or fruits from small and from large trees to realize that in these larger, woody plants, at least, there is no very striking correlation between the size of the body and the size of its parts. In certain herbaceous forms, however, evidence has from time to time been brought forward that such a correlation does in fact exist and that the largest plants (whether measured by dry weight, height, number of stalks or yield) are those which bear the largest fruits and seeds. The importance of such a conclusion on the theory and practice of seed selection is evident; and the

[1] This investigation was carried on by the aid of a grant from the American Association for the Advancement of Science.

question is also of considerable theoretical significance in that it bears directly on the perplexing problems of individuality and organization. The aim of the present paper is to contribute to the solution of this problem by undertaking a careful analysis and interpretation of size relations in a series of bean plants.

HISTORICAL

The problem, at least in certain of its aspects, has received attention at the hands of workers in several fields. Students of the cereal grains, in particular, have been interested in determining whether those plants which are large in the sense of having tall or many stems or a high yield are plants which produce large heads and seeds. This question is of importance in seed selection, since if high yield and large seed size are correlated, it will be comparatively easy to pick out from a mixture those seeds which have been produced by high-yielding plants. Scattered papers on other crops than the cereals also provide facts of interest.

Most work has been done with the small grains, particularly wheat and oats. Lyon (11) in 1906, although not using biometrical methods, observed that in wheat the weight of the average kernel is not correlated with the number of kernels per head or with the number of kernels per plant. He states that the highest yielding plants have medium-sized spikes and medium-sized kernels.

Waldron (20) in 1910, working with oats, reported substantial *negative* correlations ($-.4$ to $-.6$ approximately) between average weight of seed per plant and (1) number of seeds, (2) length of head, and (3) length of culm, thus indicating that the larger the plant, the smaller were its seeds.

Results at variance with those of Waldron were recorded in 1911 by Love (9), Roberts (14) and Myers (12), working with wheat, and by Leighty (7), with oats.

These authors generally agree that there is a positive, though small, correlation, usually of about the magnitude of .2 to .4, between the size of the plant, as measured by height or by yield, and the average weight of the seeds it produces. Larger plants also tend rather consistently to have larger heads.

In two extensive memoirs on oats in 1914 Love and Leighty (10) and Leighty (8) presented an abundance of data on this problem. In the former paper the authors find positive and fairly large correlations between the size of the plant, as measured by yield, and the size of each head, as measured by its individual yield or by the number of spikelets or number of kernels which it produces. The average weight of kernel per plant is not very consistently correlated with any character representative of plant size, however, although most of the correlation coefficients are positive and many are, in certain seasons, significantly large. In the second paper the author, working with another variety, finds consistent, positive and significant correlations between plant height and average weight of kernels. He points out that the degree of correlation in all characters studied increases considerably as the plants are reduced in size through crowding.

Arny and Garber (1) in 1918 found that, in wheat, plant height and plant yield are positively correlated with spike length; and that average kernel weight is positively and consistently correlated with yield (total kernel weight) and with number of kernels. The authors mention unpublished work of Atkinson and Hutchinson who found substantially the same results.

With corn, the reports are somewhat conflicting. Ewing (2) found a positive but small correlation between yield and leaf length and breadth. Hutcheson and Wolfe (6) found a significant correlation between yield and both length and circumference of ear. Olson, Bull and Hayes (13) and others, however, find no significant correlation between yield and any other character studied.

With apples, Shaw (15, 16, 18) and Stewart (19) have pointed out that there is little relation between the yield of fruit on a tree and the average size of the fruit except in very heavy yields, when fruit size decreases. Small, young trees may have slightly larger fruits than large, old trees. Many investigators maintain, however, that under most conditions thinning will increase the size of the fruit and thus imply that there is usually a negative correlation between yield and fruit size.

In tobacco, Hayes (5) found that there is probably no significant correlation between number of leaves and average leaf area.

In beans, Harris (3, 4) found a small positive correlation between number of pods per plant and (1) number of ovules per pod and (2) average weight of seed per plant, the higher yielding plants thus having somewhat larger pods and somewhat heavier seeds.

In peas, Shaw (17) found that a positive but small correlation exists between length of vine and average weight of seed produced, and that this correlation is much greater in small plants than in large ones.

The total evidence is therefore conflicting. A majority of the workers report positive correlations between plant size and the size of the various organs produced. These correlations, however, even when significant, are in most cases so small, and there are so many instances where the coefficients are clearly not significant or are even negative, that no general conclusion, supported by the whole body of evidence, can well be drawn.

MATERIALS AND METHODS

The present paper is the result of a study of a group of 562 bean plants grown during the summer of 1918 as a part of a larger investigation. The beans were Red Kidneys, and although they were not members of a pure line, they were so similar in all characters studied as to indicate that no wide genetic differences existed among

them. Seeds of uniform size were selected and were planted June first. The soil of the plot varied decidedly in fertility, with the result that some of the plants grew luxuriantly, many of them reaching a total dry weight of 150 grams, whereas others were much dwarfed, reaching only 4 or 5 grams at maturity. The bulk of the population were intermediate in size.

Over two hundred of the plants were harvested from time to time during the summer, representatives of all stages from the young seedling to the appearance of flowers being obtained. The rest, 344 in number, grew to maturity and were harvested then. The number of leaves,[2] pods and seeds were counted and dry weight determinations made of the total bulk of the leaves, of the stem system and of the yield of fruit, separate determinations being recorded for total pods (without seeds) and total seeds. From these data, the dry weight of the entire plant, of the vegetative shoot and of the reproductive structures could easily be determined, as well as the average weight of leaf, pod and seed for each plant. Correlations were then made between the average weight of each of these organs, respectively, and the size of the plant. The latter was represented either by the weight of the shoot (stem plus leaves), the weight of the fruit (yield), the number of leaves, the number of pods or the number of seeds.

Results

The coefficients of correlation thus determined are set forth in Table I. Eight of these involve the 344 mature plants studied and one (the first) involves the 218 immature plants. Three of the correlation tables on which these constants are based—those showing the relation between shoot weight and (1) average leaf weight, (2) average pod weight and (3) average seed weight in mature plants—are also presented in Tables II, III and IV.

It will be noted that in every case there is a positive

[2] Very small leaves, less than one third the average size for the plant, were not counted.

TABLE I

CORRELATIONS BETWEEN BODY SIZE AND AVERAGE ORGAN SIZE

Dry weight of shoot: Average dry weight of leaf[5]	$r = +.891 \pm .009$
Dry weight of shoot: Average dry weight of leaf	$r = +.769 \pm .015$
Number of leaves: Average dry weight of leaf	$r = +.607 \pm .023$
Dry weight of shoot: Average dry weight of pod	$r = +.301 \pm .033$
Total weight of fruit: Average dry weight of pod	$r = +.460 \pm .029$
Number of pods: Average dry weight of pods	$r = +.219 \pm .035$
Dry weight of shoot: Average dry weight of seed	$r = +.229 \pm .035$
Total weight of fruit: Average dry weight of seed	$r = +.390 \pm .031$
Number of seeds: Average dry weight of seed	$r = +.180 \pm .035$

correlation between the average weight of the organ studied and the size of the entire plant, however measured. This correlation is in most cases rather small in amount, but in every instance but one the coefficient is more than six times as large as its probable error and may therefore be regarded as significant. The coefficients are least in the case of the seed, somewhat higher for the pod and of considerable magnitude for the leaf, being particularly high in the case of the immature plants. From these figures, therefore, it might reasonably be inferred that there is a significant relation, though a small one, between organ size and size of plant in beans,

TABLE II

CORRELATION BETWEEN DRY WEIGHT OF SHOOT AND AVERAGE DRY WEIGHT OF LEAF

Dry weight of shoot in grams (Class centers)

Average dry weight of leaf in grams (Class centers)	2	6	10	14	18	22	26	30	34	38	42	46	50	54	58	62	66	70	74	78	82	86	90	94
1.25														1										
1.15										1	1								1					
1.05																								
.95									2			1	1		1	1			1					
.85							2	1	4	4	5	8	5	4	3	5	6	1	1		1			1
.75				2		6	3	8	6	6	6	5	5	4	5	3	1							1
.65			1	1	4	7	13	10	5	5	1	5	1	2		1					1			
.55		1	2	4	11	17	9	3	3	1	1	1	2		2			1						
.45		2	8	18	3	1	6		1		1					1								
.35		11	15	3	1																			
.25	3	16	3																					
.15	6	6																						
.05	1																							

$r = +.769 \pm .015$

(For plants below 40 grams in shoot weight, $r = +.842 \pm .013$, for plants above 40 grams, $r = +.129 \pm .067$.)

[5] The group of immature plants only.

TABLE III

CORRELATION BETWEEN DRY WEIGHT OF SHOOT AND AVERAGE DRY WEIGHT OF POD

Average dry weight of pod in grams (Class centers)	Dry weight of shoot in grams (Class centers)																					
	2	6	10	14	18	22	26	30	34	38	42	46	50	54	58	62	66	70	74	78	82	86	90	94
2.75				1																				
2.65																								
2.55						1								1			1							
2.45							1							1										
2.35								1				1			1	2								
2.25	1							1	1		1						1							
2.15					1		1	1	1						1		1							1
2.05				2	1		2	1	2	1			2			1								
1.95					8	7	2	2	1	4	1	1		1	1					2	1			
1.85				1	5	2	6	6	2	1	2	1	3		2		2	3			1			
1.75	1	1		4	3	1	4	5	2	4	4		4	2		1	1	1						
1.65			4	4	3	3	6	5	5	1	4	5	3	6	2	3	2	1						
1.55			4	8	3	3	5	6	3	1	4	3	3	2	1	4	1			1		2		
1.45			5	1		1	2		2	3	1	2	2	1	1		2	2						1
1.35			9	2	3	1	1	4	2	3	1		1		2									
1.25			5	4	1		2	2		1		2	1											
1.15	1	5	3																					
1.05	1																							
.95	2	1																						
.85	2																							
.75	2	1																						
.65		1																						

$$r = + .301 \pm .033$$

(For plants below 16 grams in shoot weight, $r = + .591 \pm .043$; for plants above 16 grams, $r = + .050 \pm .044$.)

the larger plants producing larger leaves, pods and seeds and *vice versa*. This conclusion agrees with that of most previous workers.

A more intensive study of the correlation tables, however, reveals certain facts which do not harmonize with this conclusion, and which suggest that the whole problem is somewhat too complicated to be solved merely by determining such a series of correlation coefficients as appear in Table I. A study of the curves connecting the means for organ size in the correlation tables from which these constants have been derived shows that regression is far from linear and that the character of the curve is essentially the same in every case. The eight curves for the means of organ size on body size in the mature plants are shown in Fig. 1. In every case it is clear that as we

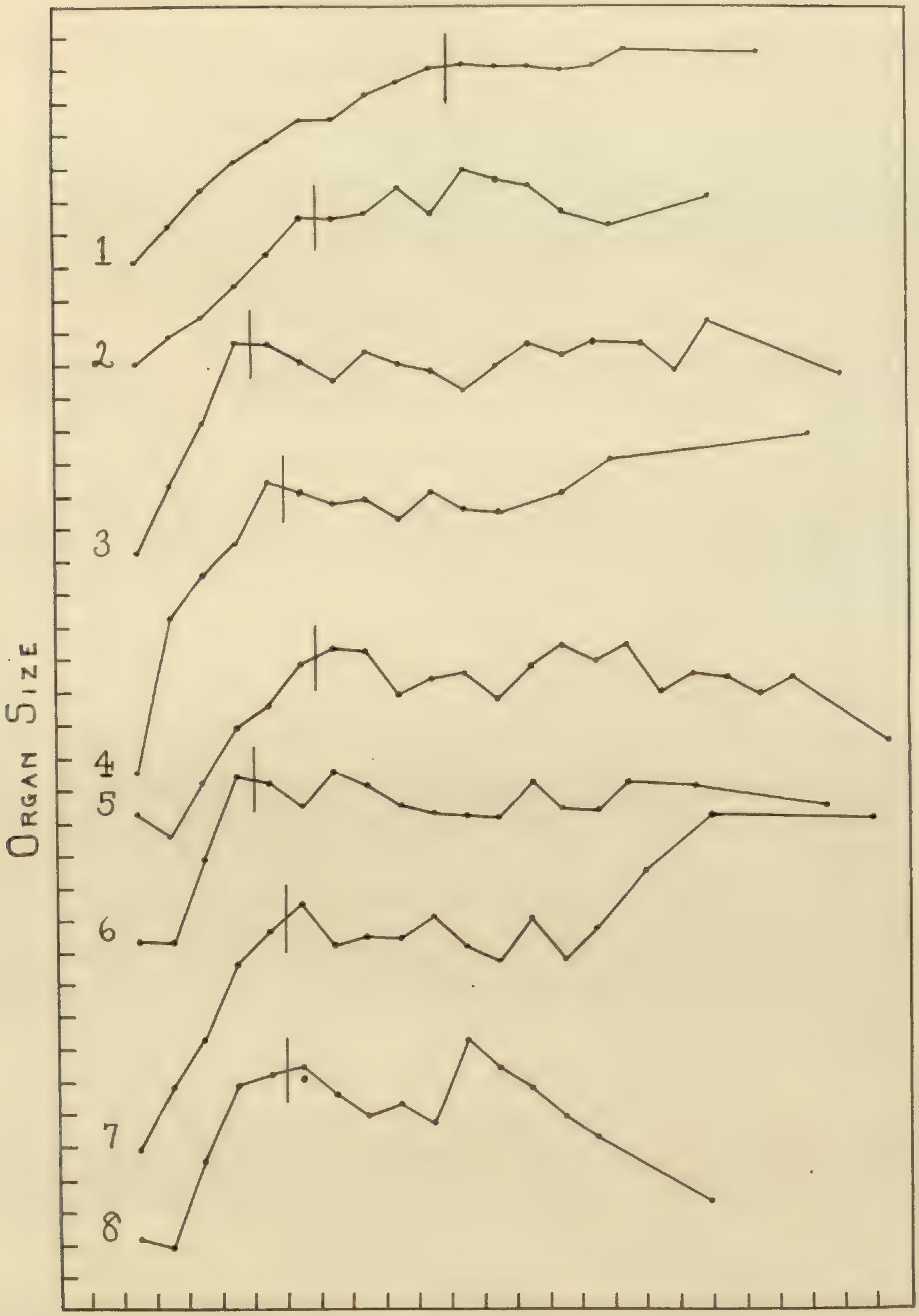

FIG. 1. Curves of the means for organ size on body size in mature plants. (1) Leaf weight on shoot weight; (2) leaf weight on number of leaves per plant; (3) pod weight on shoot weight; (4) pod weight on yield of fruit; (5) pod weight on number of pods per plant; (6) seed weight on shoot weight; (7) seed weight on yield of fruit; seed weight on number of seeds per plant. Vertical lines indicate points where division was made into "large" and "small" plants.

progress from the smaller plants to the larger ones there is *up to a certain point* a marked increase in the average organ size for the plant; but that beyond this point the curve flattens and thence onward an increase in plant size has essentially no effect on the average size of leaf, pod or seed.

TABLE IV

CORRELATION BETWEEN DRY WEIGHT OF SHOOT AND AVERAGE DRY WEIGHT OF SEED

Dry weight of shoot in grams (Class centers)

Average dry weight of seed in grams (Class centers)	2	6	10	14	18	22	26	30	34	38	42	46	50	54	58	62	66	70	74	78	82	86	90	94
.530								1					1			1								
.515							1																	
.500					1						1		1		1									
.485														1										
.470			1			1	2		1			1			1	1								
.455			1	3		1		1	1															
.440					1		2					1		1										
.425						2	1	1		1						1	1	1						
.410			1	5	3	1	4		2	2	1	3		1		1	2	1						
.395	2		2	4	2	5	1	2	3		1	1	1											1
.380			1	7	3	7	8	8	4	3	2	1	3	2	1	1			1		1			
.365	1	1	1	1	1	3	2	3	1	3	3	3	2	1	2	2	1							1
.350		2	7	2	6	3	8	1	2	3	2	3	3	1	3		1		2					
.335		3	2	2		2	1	1	2	4	1	2	1	1	1	1								
.320	1	11	8	3	1	2	2	2	3		3	2	1				1				1			
.305		5	1			1		1					1	1	1		1							
.290	1	4	1	1		1	1	1	1	1		2		1		3								
.275	1	6			1						1			1										
.260	2	1	1			1			1			1			1									
.245	1	1				1																		
.230		1	2																					
.215	1																							
.200																								
.185																								
.170		1																						

$r = + .229 \pm .035$

(For plants below 16 grams in shoot weight, $r = + .555 \pm .046$; for plants above 16 grams, $r = - .030 \pm .043$.)

There is thus evidently a much higher correlation between body size and organ size in small plants than in larger ones, and a single correlation coefficient covering both groups clearly fails to give an accurate picture of the facts. Each of the eight correlations involving the mature plants was therefore divided into two parts, one including the small plants and one the large ones, the line of division coming at approximately the point where

the curve of means stops ascending and begins to flatten out. This point is marked by a vertical line on each of the curves in Fig. 1 and in the same way in Tables II, III and IV. The coefficient of correlation was now determined both for the group below this point and for the group above it—for the small plants and for the large ones. The constants thus derived are shown in Table V. A study of these figures makes it clear that in the smaller plants there is a decided correlation between body size and organ size (always exceeding ten times its probable error), but in the larger plants practically none at all.[3] This emphasizes the conclusion drawn from the character of the curve of means, namely, that up to a certain point increased body size is followed by increased size of organs produced, but that beyond this point there is no relation between the two.

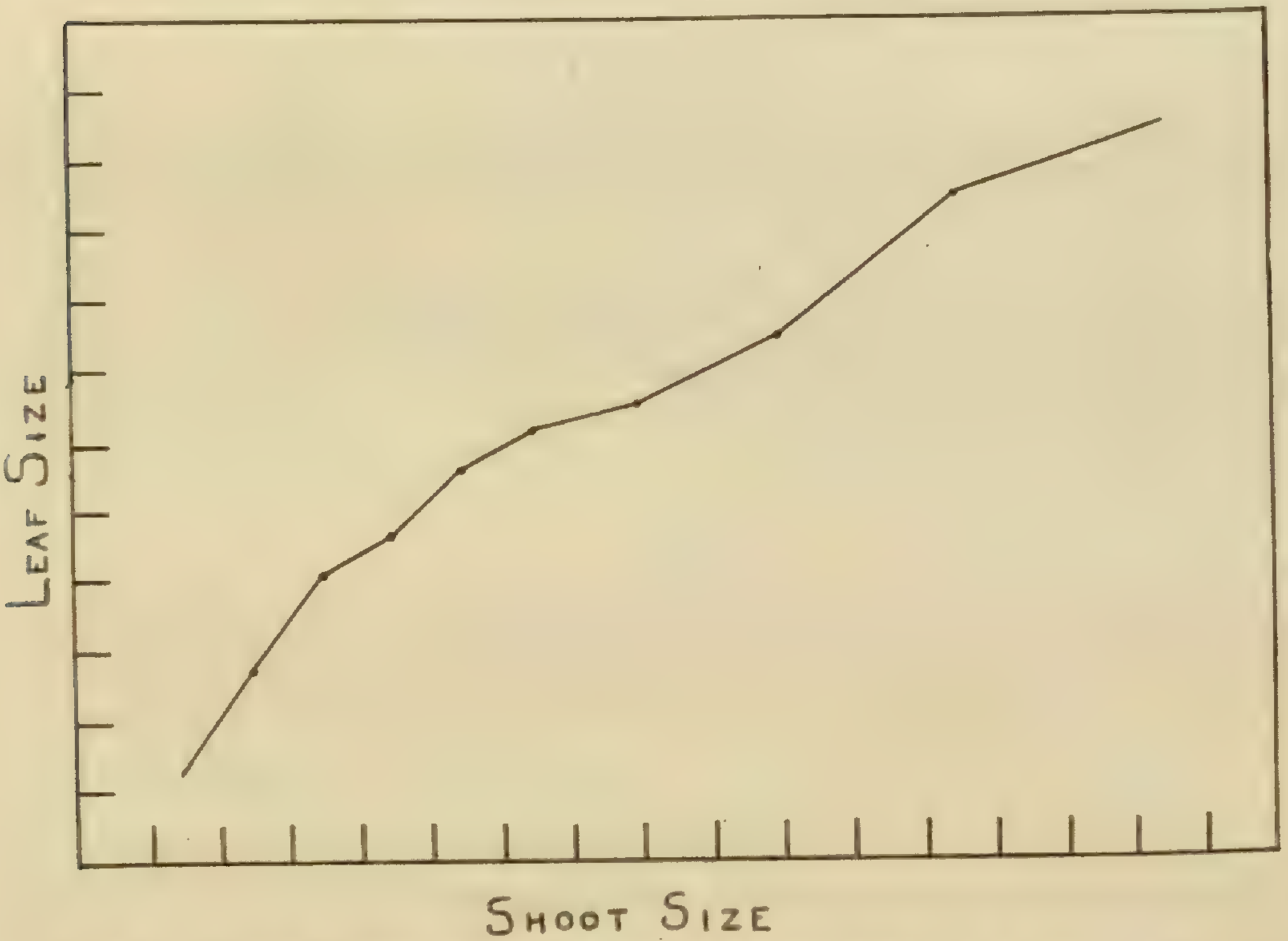

FIG. 2. Curve of means for leaf weight on shoot weight in immature plants.

It is of interest to note that in the case of the immature plants the curve of means for leaf size rises steadily as

[3] It will be noted that very similar results were obtained by Shaw (17) in correlating seed size with vine length in peas.

plant size increases (Fig. 2), showing no sign of the flattening characteristic of the mature plants.

What reason may we assign in the case of the mature plants for this radical difference between large individuals and small ones? And why, in immature plants, should no such difference exist? The suggestion at once comes to mind that there may really be no relation between body size and organ size in any case, but that organ size may be determined, instead, by the size of the particular axial *growing point* from which the organ has

TABLE V

CORRELATIONS BETWEEN BODY SIZE AND AVERAGE ORGAN SIZE IN THE ENTIRE GROUP (OF MATURE PLANTS); IN THE SMALLER PLANTS (THOSE BELOW THE VERTICAL LINE IN FIG. 1); AND IN THE LARGER PLANTS (THOSE ABOVE THE VERTICAL LINE IN FIG. 1).

	Entire Group	Smaller Plants	Larger Plants
Shoot: Average leaf	+.769±.015	+.842±.013	+.129±.067
Number of leaves: Average leaf	+.607±.023	+.665±.029	+.111±.050
Shoot: Average pod	+.301±.033	+.591±.043	+.050±.044
Total fruit: Average pod	+.460±.029	+.652±.040	+.279±.040
Number of pods: Average pod	+.219±.035	+.486±.048	−.083±.045
Shoot: Average seed	+.229±.035	+.555±.046	−.030±.043
Total fruit: Average seed	+.390±.031	+.559±.047	+.206±.041
Number of seeds: Average seed	+.180±.035	+.509±.045	−.059±.045

developed. It is a matter of common observation that in most herbaceous plants the diameter of the newly formed stem internodes (and therefore presumably the diameter of the terminal growing point which gives rise to the primary tissues of the stem) is comparatively small in the seedling, but increases slowly as the plant grows larger until a presumably optimum diameter is attained which is rarely exceeded except in the case of very rank and luxuriant shoots.[4] Further growth of the plant as a whole results in an increase in the length and number of its stems, but in no increase in their primary diameter. Stem diameter is of course not uniform, many lateral

[4] The thickness of these primary tissues of the young stem is of course increased later by secondary growth, with which we are not concerned.

branches and even the main ones under unfavorable conditions, or as vegetative growth slackens, being comparatively small; and we know that organ size also varies considerably in the individual. The point to be emphasized here is that there tends to be a maximum for the primary diameter of the stem which is attained while the plant is still fairly small, and which thereafter is normally not exceeded, no matter how great the total size of the plant body may eventually become. The same rule applies of course to woody plants, the twigs of a large tree being no thicker, other things being equal, than those of a small tree, although both are usually thicker than the early axis of the seedling.

Now if the organs (leaves and fruits) developed by the primary meristem owe the size which they finally attain to the size of the growing point from which they arise; or if, to put it another way, all the structures developed at a given growing point—the stem axis and the lateral organs—are correlated with one another in size, then the biometrical results which we have set forth in our bean plants are readily explicable. The comparatively small plants are, on this supposition, the ones which did not attain at maturity sufficient size to have arrived at the maximum stem (growing point) diameter; and the smaller the plants, the smaller is their stem diameter, down to depauperate individuals whose mature primary axes are no stouter than those of the seedling. In these smaller plants, therefore, the significant correlation which we observed would naturally be expected between organ size (dependent on growing point size) and body size (definitely related to growing point size). In the case of the larger plants, however (those above the point at which the curve of means flattened out), where the maximum stem diameter or growing point size has already been attained and where, therefore, there is no relation between body size and growing point size, it is only natural that there should be (as we observed) no correlation at all. Furthermore, in the group of imma-

ture plants studied, which included everything from seedlings to plants coming into flower, there is naturally a very close relation between body size and growing point size, since these individuals all belong to that portion of the plant's life history where its primary growing points are progressively increasing in diameter, the maximum being attained in beans just before the blooming period. It would be only natural that in this group of plants in which both body size and growing point size are progressively rising, there should be a high correlation between body size and organ size.

This hypothesis of a direct relation between the size of an organ and the size of the growing point from which it arose will evidently explain the facts which we have observed in the case of our bean plants. The problem now remains to discover a means whereby we may determine more directly the soundness of the hypothesis. The size of an organ can be measured fairly accurately either by weighing it or by determining its dimensions and computing its volume. To get a measurement which shall represent at all adequately the size of the growing point, however, is a more difficult matter. The cross-sectional area of the stem axis which is produced from the growing point might be used; but since the terminal growing point is of course a primary meristem entirely and since the early activity of a secondary growing point or cambium often affects almost immediately the diameter of the stem, it is evident that stem cross section, particularly in regions very far removed from the growing point, can not be counted on as an accurate indication of growing point size. We can confine ourselves to a tissue which is entirely primary in its origin, however, and which is not affected by subsequent secondary growth if we measure simply the *pith*. The magnitude of the correlation between organ bulk and cross-sectional area of the pith of the internode below the attachment of the organ might be expected to give us a fairly good idea as to whether organ size and grow-

ing point size are definitely related to one another or not. The organ most readily studied and most clearly significant in such a problem is of course the leaf.

The bean plant is evidently not well suited for such a study, since its pith is rather irregular in outline and not sharply delimited. The twigs and leaves of the rock maple (*Acer saccharum*), however, on which the writer is carrying on some other work, have proven very satisfactory for an investigation of this kind. The pith in this species is approximately circular in cross section and is sharply delimited, and the leaves are of uniform and fairly considerable thickness.

A series of twigs collected during the summer from a single tree were studied. The area of the blade was determined by tracing its outline on standard weight paper, cutting this out and weighing the cut-out. Blade thickness was measured by a micrometer caliper at two points away from the main veins and situated symmetrically on opposite sides of the midrib, the average of the two measurements being taken. The product of area X thickness of course gives us the blade volume. To determine pith area a cross section was cut at the middle of the internode. the pith diameter measured in two directions at right angles to each other by a micrometer stage, the results averaged and the area computed therefrom.

Total blade volume (the sum of the volumes of the two blades borne at a given node) was correlated with the cross-sectional area of the pith of the internode below for over 100 leaf pairs from this tree, taken from all parts of its crown. The results are shown in Table VI. It is quite evident from the size of the correlation coefficient ($+.807 \pm .024$) that there is an unquestionable relationship between leaf size and pith area, the size of the leaf being governed pretty largely by the stoutness of that portion of the twig from which it springs. It would seem, therefore, that the size relationships between the structures laid down by the terminal meristem persist as these structures develop to maturity, these *relative* sizes re-

maining constant regardless of the *actual* size which is attained. The terminal growing point, like the animal embryo, develops as a symmetrical and interrelated whole.

Although the size of the leaf thus seems to be closely dependent upon that of the growing point, the size of the reproductive organs is evidently much less so. We have

TABLE VI

CORRELATION BETWEEN THE BLADE VOLUME (IN CUBIC CENTIMETERS) OF THE TWO LEAVES AT A NODE, IN ACER SACCHARUM, AND THE CROSS SECTIONAL AREA (IN SQUARE MILLIMETERS) OF THE PITH OF THE INTERNODE BELOW.

Blade volume in cubic centimeters (Class centers)

Pith area in square millimeters (Class centers)	.3	.5	.7	.9	1.1	1.3	1.5	1.7	1.9	2.1	2.3	2.5	2.7	2.9	3.1	3.3	3.5	3.7	3.9	4.1	4.3	4.5	4.7
7.05																						1	
6.75																							
6.45																							
6.15																							
5.85																							
5.55																							
5.25																					1		1
4.95																						2	
4.65																1				2	1		
4.35																						1	
4.05													1									1	
3.75													1	1				1					
3.45					1	1								1	2								
3.15									1													1	1
2.85							1					1	1	1	1			1			1		
2.55					1							1		2	1	1							
2.25						1									3	1			1				
1.95							1		3			1											
1.65						2			1		1	3	1	1									
1.35		1		1	1			2		2	2			1									
1.05			2	3		1	5	1	2		1												
.75	1	1		2	2	1	3	3															
.45			2		3	1																	
.15			2																				

$r = + .807 \pm .024$

seen that leaves do not reach their maximum size except in plants with shoots of about forty grams dry weight or more. The maximum pod and seed size, however, is attained in much smaller plants, usually those in the vicinity of sixteen grams. In other words, a reduction in the size of the growing point is felt much more quickly by the leaf than by the fruit. It is only in plants which are really depauperate that the fruit and seed size is reduced

below the normal. The reason for this may lie in the fact that the flower is an independent axis rather than a lateral organ like the leaf and may therefore be less affected by the size of the main axis from which it springs. It is well known that flowers are more constant in size and other characters and less affected by environmental conditions than are vegetative organs.

It should be recognized that in the grasses, where most of the work along this line has been done, conditions are somewhat different from those in dicotyledons such as the bean; since the plant body, or at least each shoot or culm, is essentially determinate in growth, with a limited number of parts. This may sometimes affect the statistical results obtained, but we believe that conditions are fundamentally the same in the two groups. It is of interest to note the conclusions of Leighty and others, for small grains, *viz.*, that the correlations between organ size and plant size are considerably higher in small, poorly developed plants than in large ones,—a situation precisely similar to that which we have reported in beans. The whole problem can perhaps be solved best, however, by studying such an indeterminate type of plant body as is characteristic of the ordinary dicotyledon.

Conclusions

We may conclude, therefore, that the size of the plant body is not the direct causative factor in determining the size of the leaves, fruits or seeds which it produces, as has been suggested or implied by many investigators, but that the size of any given organ depends rather upon the size of the growing point out of which it has been developed. Any factor, be it age, moisture or food supply, which alters the size of the meristem, will thus alter the size of the organs produced by this meristem. There seems to be nothing in these higher plants closely corresponding to the definite organization with which we are familiar in the animal individual, where size of body is definitely related to size of organs.

The present study emphasizes the difficulties lying in the path of the investigator who attempts to solve such a problem as this merely by determining a single series of biometrical constants without taking into account the various morphological and physiological factors which may be involved.

Summary

1. The problem of the relationship between the size of the plant body and the size of the organs it produces has been studied by various workers, who find that in most cases there is a small but significant correlation between these characters.

2. In a series of bean plants, the coefficients of correlation were determined between plant size, as measured by dry weight of shoot, dry weight of fruit, number of leaves, number of pods and number of seeds; and the average dry weight per plant of leaf, pod and seed. A positive and significant correlation, though usually a small one, was found in each case.

3. An examination of the curve of means for organ size on plant size shows that in each case the curve rises steeply at first and then flattens out. In other words, an increase in the size of the plant is accompanied by an increase in the size of its organs if we consider comparatively small plants only; but after a certain size is reached, any further increase in plant size is not followed by increase in organ size. Separate correlations between plant size and organ size made for small plants (those below the point where the curve flattens) and for large ones (those above it) showed a very decided correlation in the former and practically none at all in the latter.

4. These facts suggested that the size of an organ may not be correlated with body size at all, but rather with the size of the axial growing point from which it develops. In support of this idea attention is called to the fact that during the early stages of a plant's growth there is

up to a certain point a progressive and parallel increase in the size of the plant and in the size of the primary meristems of its axes; but that after this point is reached meristem size remains constant and further increase in body size is not accompanied by any increase at all in that of the growing point.

5. Favorable material to test this hypothesis directly was afforded by twigs and leaves of *Acer saccharum*. The correlation between the blade volume of a given leaf pair and the cross-sectional area of the pith of the internode below (used as an index to the size of the growing point from which the leaves had developed) was found to be high ($+.807 \pm .024$).

6. It is therefore concluded that the size of a plant organ (leaf, fruit or seed) is dependent not upon the body size of the plant on which it is borne, but rather upon the size of the growing point from which it developed.

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DECREASE IN SEXUAL DIMORPHISM OF BAR-EYE DROSOPHILA DURING THE COURSE OF SELECTION FOR LOW AND HIGH FACET NUMBER[1]

PROFESSOR CHARLES ZELENY

UNIVERSITY OF ILLINOIS

IN the bar-eye race of *Drosophila* the mean eye facet number of the males is higher than that of the females, though there is an overlapping in range as shown in Table I. In the unselected white bar stock used as the starting point of a series of selection experiments this difference amounted to 6.12 factorial units, a factorial unit being one that produces a ten per cent. change in facet number. If this value were fairly constant it would be possible in treating the selection data statistically to reduce the facet values of the two sexes to a common basis in much the same way that Galton obtained a mid-parental value in his studies of the inheritance of human stature. Such a procedure was followed by Zeleny and Mattoon (1915)[2] in their paper on selection in red bar-eye. In attempting to apply the same method to the white bar series it was discovered that the difference between the two sexes is not constant but decreases during the course of selection. It is therefore not practicable to apply a constant coefficient for the reduction of the value of one sex to that of the other.

The selection experiment in question started with a white bar stock which had been obtained by crossing white full-eye to bar-eye. Single pair, brother and sister, matings were adhered to with a few breaks due to sterility. In the low line the lowest available virgin female

[1] Contribution from the Zoological Laboratory of the University of Illinois, No. 187.

[2] Zeleny, C., and Mattoon, E. W., 1915, "The Effect of Selection upon the 'Bar Eye' Mutant of *Drosophila*," *J. Exp. Zool.*, 19: 515–529.

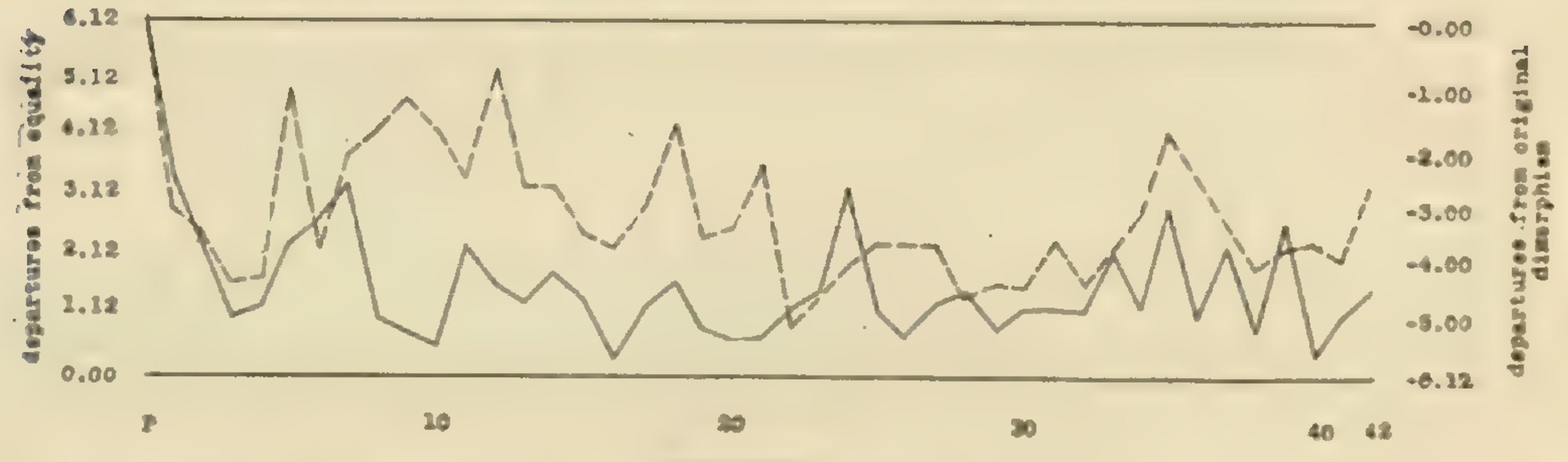

FIG. 1. Sexual dimorphism in each of the selection generations. Direct matings only. The difference between males and females is expressed in factorial units, a factor of unit value being one capable of producing a ten per cent. change in facet number. In the unselected population this difference amounts to 6.12 units and in the figure such a value is represented by the upper horizontal line. A zero difference between the sexes is represented by the lower horizontal line. The scale of departure from equality is shown at the left hand and the scale of departure from the original value in the unselected population at the right hand.

was mated to her lowest available brother in each generation with duplicate matings to insure the continuation of the line. In the same way in the high line the female with the highest facet count was mated to her highest brother.

The data as given in the present paper are grouped under two heads. In Table II and Fig. 1 are included only those individuals in the direct line. In Table III and Fig. 2 there are included, in addition, the sib matings in each generation. The general results obtained from the two groupings are alike, but the data based on all the matings give a smoother curve because of the larger numbers of individuals involved.

Table I shows the distribution of frequencies of factorial values for eye facet number in the unselected population of white bar, number 127, which served as the

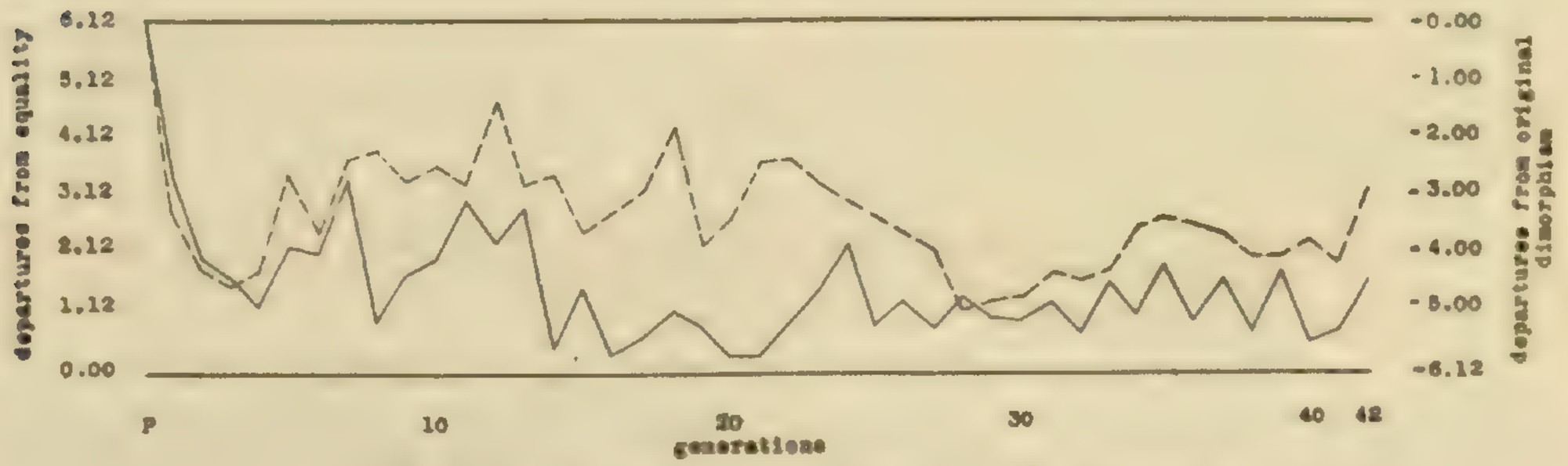

FIG. 2. Sexual dimorphism. All matings.

TABLE I

DISTRIBUTION OF FREQUENCIES FOR FEMALES AND MALES IN THE UNSELECTED WHITE BAR POPULATION AND IN THE FORTIETH GENERATION OF LOW AND HIGH SELECTION FOR EYE FACET NUMBER

Each class has a range equal to ten per cent. of its mean.

Facets in Each Class	Classes in Factorial Units		Unselected Population		F40			
					Low		High	
	♀	♂	♀	♂	♀	♂	♀	♂
359–396.....	+19.07	+12.95						
325–358.....	+18.07	+11.95						
294–324.....	+17.07	+10.95		1				
266–293.....	+16.07	+ 9.95	1					
241–265.....	+15.07	+ 8.95		3				2
218–240.....	+14.07	+ 7.95		6				5
197–217.....	+13.07	+ 6.95		15				17
178–196.....	+12.07	+ 5.95		16			1	20
161–177.....	+11.07	+ 4.95		26			8	21
146–160.....	+10.07	+ 3.95		19			12	14
132–145.....	+ 9.07	+ 2.95	1	34			23	20
119–131.....	+ 8.07	+ 1.95		35			12	2
108–118.....	+ 7.07	+ 0.95	7	33			9	2
98–107.....	+ 6.07	− 0.05	11	50			4	
89–97	+ 5.07	− 1.05	27	55				
81–88	+ 4.07	− 2.05	25	37				
73–80	+ 3.05	− 3.05	29	29				
66–72	+ 2.07	− 4.05	51	23				
60–65	+ 1.07	− 5.05	55	22				
54–59	+ 0.07	− 6.05	54	18	1	7		
49–53	− 0.93	− 7.05	58	8	8	3		
44–48	− 1.93	− 8.05	54	7	19	34		
40–43	− 2.93	− 9.05	47	2	33	24		
36–39	− 3.93	−10.05	40	1	21	9		
33–35	− 4.93	−11.05	15	1	2			
30–32	− 5.93	−12.05	9		1			
27–29	− 6.93	−13.05	3					
24–26	− 7.93	−14.05	1					
22–23	− 8.93	−15.05						
20–21	− 9.93	−16.05	1					
Totals.....			489	441	85	77	69	104

basis of the present selection series, and of the fortieth generation of low and of high selection for eye facet number. The column at the extreme left gives the facet values of the classes. The second and third columns give the factorial values of these classes, a unit of value being a difference in germinal or environmental factors which produces a ten per cent. change in facet value. The mean values of the unselected population are taken as zero and in the second column there are given the values on this scale for the females and in the third column for the

males. It will be noticed that there is considerable variation in both females and males, but the mean values as represented by the figures in heavy type are a consider-

TABLE II

SEXUAL DIMORPHISM. DIRECT LINE

Sexual dimorphism for each of the 42 generations of selection for low and high facet number. All values are in ten per cent. factorial units. Such a unit is any difference in germinal or environmental factors which produces a ten per cent. change in facet number.

Generation	Low Line		High Line		Difference in Dimorphism Between High and Low Lines
	Dimorphism	Total Change in Dimorphism	Dimorphism	Total Change in Dimorphism	
P.....	6.12	0.00	6.12	0.00	0.00
1.....	3.34	−2.78	2.74	−3.38	−9.60
2.....	2.25	−3.87	2.36	−3.76	0.11
3.....	0.95	−5.17	1.54	−4.58	0.59
4.....	1.12	−5.00	1.61	−4.51	0.49
5.....	2.20	−3.92	4.89	−1.23	2.69
6.....	2.61	−3.51	2.13	−3.99	−0.48
7.....	3.26	−2.86	3.82	−2.30	0.56
8.....	0.92	−5.20	4.17	−1.95	3.25
9.....	0.69	−5.43	4.74	−1.38	4.05
10.....	0.48	−5.64	4.17	−1.95	3.69
11.....	2.17	−3.95	3.38	−2.74	1.21
12.....	1.49	−4.63	5.28	−0.84	3.79
13.....	1.20	−4.92	3.20	−2.92	2.00
14.....	1.67	−4.45	3.22	−2.90	1.55
15.....	1.26	−4.86	2.41	−3.71	1.15
16.....	0.21	−5.91	2.16	−3.96	1.95
17.....	1.13	−4.99	3.33	−2.79	2.20
18.....	1.51	−4.61	4.29	−1.83	2.78
19.....	0.78	−5.34	2.31	−3.81	1.53
20.....	0.55	−5.57	2.45	−3.67	1.90
21.....	0.59	−5.53	3.58	−2.54	2.99
22.....	1.08	−5.04	0.79	−5.33	−0.29
23.....	1.38	−4.74	——	——	——
24.....	3.17	−2.95	1.87	−4.25	−1.30
25.....	1.05	−5.07	2.18	−3.94	1.13
26.....	0.61	−5.51	0.57	−5.55	−0.04
27.....	1.14	−5.98	2.19	−3.93	1.05
28.....	1.35	−4.77	1.24	−4.88	−0.11
29.....	0.75	−5.37	1.46	−4.66	0.71
30.....	1.04	−5.08	1.40	−4.72	0.36
31.....	1.07	−5.05	2.29	−3.83	1.22
32.....	1.01	−5.11	1.46	−4.66	0.45
33.....	1.97	−4.15	2.09	−4.03	0.12
34.....	1.03	−5.09	2.67	−3.45	1.64
35.....	2.75	−3.37	4.11	−2.01	1.36
36.....	0.83	−5.29	3.22	−2.90	2.39
37.....	2.10	−4.02	2.13	−3.99	0.03
38.....	0.71	−5.41	1.79	−4.33	1.08
39.....	2.53	−3.59	2.08	−4.04	−0.45
40.....	0.26	−5.86	2.20	−3.92	1.94
41.....	0.95	−5.17	1.93	−4.19	0.98
42.....	1.37	−4.75	3.24	−2.88	1.87

able distance apart, six class units or, to be more exact, 6.12 as stated in the first paragraph. In both low and high selection lines it is to be noticed that the variability has decreased and at the same time the mean values of females and males have approached each other. This approach is greater in the low than in the high line.

Tables II and III give the sexual dimorphism for the parental unselected generation and for each of the 42 generations of selection. Each table includes, for both low and high lines, the value of the dimorphism for each generation and the decrease since the beginning of selection. In the last column of each table there is a comparison of the dimorphisms in the two selection lines. Table II includes only the offspring in the direct line. Table III gives the data for the same series, but includes sib matings as well as those in the direct line.

Figures 1 and 2 show the decrease in graphic form. The vertical scale is in factorial units as described above. The upper horizontal line is at the level of the dimorphism value of the unselected population, which is 6.12 units in favor of the males. The lower horizontal line indicates the position of zero difference between the sexes. The scale at the left gives the actual dimorphism values and the scale at the right the departures from the original value. The continuous zigzag line gives the values for each generation in the low selection series and the dotted line those in the high selection series. Special emphasis is to be laid on the fact that selection was not for low or high sexual dimorphism, but for low or high facet number regardless of dimorphism. In no sense can it be considered as a direct selection for degree of dimorphism.

The result obtained when the offspring of all matings are taken (Fig. 2) is not essentially different from that obtained in the direct line. Since it gives the smoother curve because of the larger number of individuals it will be used as the basis of the following discussion.

In the low selection line the dimorphism drops very

rapidly from its original value of 6.12 units to 1.12 in the fourth generation. From that point on it shows some increase and fluctuates irregularly for a number of gen-

TABLE III

SEXUAL DIMORPHISM. ALL MATINGS

Sexual dimorphism for each of the 42 generations of selection for low and high facet number. All values are in ten per cent. factorial units. Such a unit is any difference in germinal or environmental factors which produces a ten per cent. change in facet number.

Generation	Low Line		High Line		Difference in Dimorphism between High and Low Lines
	Dimorphism	Total Change in Dimorphism	Dimorphism	Total Change in Dimorphism	
P.....	6.12	0.00	6.12	0.00	0.00
1.....	3.34	−2.78	2.76	−3.36	−0.58
2.....	1.95	−4.17	1.78	−4.34	−0.17
3.....	1.63	−4.49	1.45	−4.67	−0.18
4.....	1.12	−5.00	1.72	−4.40	0.60
5.....	2.20	−3.92	3.47	−2.65	1.27
6.....	2.06	−4.06	2.44	−3.68	0.38
7.....	3.36	−2.76	3.70	−2.42	0.34
8.....	0.84	−5.28	3.87	−2.25	3.03
9.....	0.68	−5.44	3.35	−2.77	2.67
10.....	0.98	−5.14	3.64	−2.48	2.66
11.....	1.99	−4.13	3.29	−2.83	1.30
12.....	1.28	−4.84	4.74	−1.38	3.46
13.....	1.88	−4.24	3.20	−2.92	1.32
14.....	0.43	−5.69	3.42	−2.70	2.99
15.....	1.42	−4.70	2.44	−3.68	1.02
16.....	0.31	−5.81	2.80	−3.32	2.49
17.....	0.61	−5.51	3.19	−2.93	2.58
18.....	1.07	−5.05	4.29	−1.83	3.22
19.....	0.81	−5.31	2.31	−3.81	1.50
20.....	0.33	−5.79	2.66	−3.46	2.33
21.....	0.33	−5.79	3.70	−2.42	3.37
22.....	0.92	−5.20	3.72	−2.40	2.80
23.....	1.42	−4.70	3.33	−2.79	1.91
24.....	2.23	−3.89	2.97	−3.15	0.74
25.....	0.83	−5.29	2.71	−3.41	1.88
26.....	1.26	−4.86	1.71	−4.41	0.45
27.....	0.75	−5.37	2.15	−3.97	1.40
28.....	1.30	−4.82	1.07	−5.05	−0.23
29.....	0.94	−5.18	1.22	−4.90	0.28
30.....	0.89	−5.23	1.32	−4.80	0.43
31.....	1.23	−4.89	1.79	−4.33	0.56
32.....	0.69	−5.43	1.60	−4.52	0.91
33.....	1.55	−4.57	1.78	−4.34	0.23
34.....	1.00	−5.12	2.51	−3.61	1.51
35.....	1.84	−4.28	2.68	−3.44	0.84
36.....	0.86	−5.26	2.58	−3.54	1.72
37.....	1.61	−4.51	2.44	−3.68	0.83
38.....	0.71	−5.41	2.01	−4.11	1.31
39.....	1.75	−4.37	2.03	−4.09	0.28
40.....	0.56	−5.56	2.32	−3.80	1.76
41.....	0.77	−5.35	1.90	−4.22	1.13
42.....	1.37	−4.75	3.24	−2.88	1.87

erations, but eventually settles down to a value not far from 1.00. This last value may therefore be taken as the normal dimorphism in a homozygous low bar population.

In the high selection line there is a similar rapid decline, in this case from 6.12 in the parental generation to 1.45 in the third selection generation. There is then a pronounced increase and fluctuation between 2.00 and 4.00 from the fifth to the twenty-third generation. After a second general decline the value remains for most of the time between 1.00 and 2.50.

While the general course of the curves is the same in the two cases, the low selection line is the more consistent and reaches the lower level.

The probable explanation of the decrease in dimorphism in both selection lines is to be sought in the fact that some of the accessory factors affecting facet number are sex-linked. The unselected population is a mixed one and accordingly has a considerable degree of heterozygosis for these factors in the females. One of the results of selection for facet number with inbreeding is a decrease in this heterozygosis, leaving the females homozygous for low facet factors in the low line and for high facet factors in the high line. As long as the population is homozygous it is probable that the degree of dimorphism does not change to any great extent because there is no disturbance of the factorial proportions. An increase on one side is accompanied by a proportionate increase on the other. The same would be true as far as the heterozygous females are concerned if the dominance of low and high factors were alike. If, however, the factors for low facet number have a higher dominance coefficient than those for high facet number it follows that selection as practised will produce a decrease in dimorphism. That this will be true in both low and high lines is made clear by the consideration that heterozygous females under the stated condition must be lower than the average of the homozygous ones in the same population. When these heterozygotes are eliminated

by selection it follows that the mean value of the females approaches that of the males.

The explanation for the general decrease seems clear enough, but the reasons for some of the details are not so evident. The dimorphism in the high selection line is higher than that in the low selection line in nearly every generation. In part this difference may be due to a faulty method of determination of the dimorphism value. If degree of dimorphism were expressed in facet numbers the high line would of course have a much greater difference between the sexes than the low line. The factorial unit method with its logarithmic scale based primarily upon the observed effects of temperature is undoubtedly a much better one than that based upon the unreduced facet numbers, though a simple ratio between the facet numbers of the two sexes might have served equally well in the present case.[3] But the correction is only an approximation and may not be great enough to obtain the most rational value.

In part, however, the difference between the two lines may be due to the greater frequency of appearance of mutant accessory factors in the high than in the low line. Such factors introduce a new heterozygosis and therefore increased dimorphism which is eliminated only by later selection. That such mutants appear is evidenced by increase in standard deviation.

The appearance of mutants and their prompt elimination by selection may account also for some of the irregular fluctuations as observed in both lines. It is possible, however, that environmental factors may exercise a differential effect and thereby change the dimorphism. In favor of this view it may be pointed out that there is some tendency for the values in the low and high lines to fluctuate together and this may be explained by the fact that the food cultures of the two lines in any generation were usually made up from the same food mass.

[3] Zeleny, C., 1920, "The Tabulation of Factorial Values," AMER. NAT., 54: 358–362.

DATA CONCERNING LINKAGE IN MICE

W. L. WACHTER

Bussey Institution

In exploiting the genetics of any plant or animal it is of great importance to breed intensively and extensively until the genetic material is exhausted. Even after the dominance or recessiveness of a certain gene has been established, there yet remain to be determined the relationships between this and other genes known to constitute the hereditary complex of that particular plant or animal. These possible relationships are allelomorphic, independent, or linked. Mendel was able to formulate certain laws of heredity from the data he obtained in his breeding experiments; but had he continued his work to include other factors he would undoubtedly have been led to predict or formulate a theory of linkage. The chromosome theory of heredity involves independent inheritance of genes which lie in different chromosomes no less than linked inheritance of those which lie in the same chromosome. It is equally important in relation to this theory to know whether two genes are linked or not linked.

A very meager amount of linkage has so far been found in mammals. Cases have thus far been observed only in rabbits, rats, and mice. In rabbits Castle (1920) has recently shown that there is a probable linkage between the factors for dilution and the English type of spotting. In rats Castle and Wright (1915), and Castle (1919), had previously shown albinism, red-eye, and pink-eye to be included in the same linkage system. In mice the genes for pink-eye and albinism were first shown to be linked by the work of Darbishire (1904), as was pointed out by Haldane, Sprunt, and Haldane (1915), who confirmed the idea by observations of their own.

A great amount of data has been obtained by breeding the house mouse. Yet a further intensive study is desirable of the mutant characters discovered, especially as regards linkage, for from such study alone can be deduced the probable localization of the genes in the chromosomes. According to Harvey (1920), the number of chromosomes in the gametes of the house mouse has been estimated by different observers at from 8 to 30, the more recent observers placing the number at from 20 to 24. One of these is probably a sex chromosome which is heterozygous in the male and homozygous in the female. In this sex chromosome is apparently located the lethal gene of the Japanese waltzing mouse reported by Little (1920). In one autosome are located the linked genes for pink-eye and albinism. In another autosome must be located the gene for agouti and its allelomorphs, non-agouti, light-bellied agouti, and yellow; to a third autosome is referred piebald spotting; to a fourth, black-eyed-white spotting; while dilution and its allelomorph, intensity, will probably have to be referred to a sixth autosomal group. Thus seven of the twenty or more chromosomes are genetically identified, leaving thirteen or more yet to be identified with visible characters or mutations yet to be discovered.

Among modifying genes in mice, Little (1915) and Dunn (1920) have shown that the "blaze" or "white-face" variation which modifies the piebald pattern is heritable. Dunn (1920) also reports a "belly-spot" to segregate independently of self and piebald, though apparent only in animals heterozygous for self and piebald. General modifying factors, increasing or decreasing the area of pigment in the black-eyed-white and piebald patterns, are also heritable. Very little is known at the present time concerning these modifying factors, and nothing about their linkage relations.

THE RELATION OF AGOUTI TO PIEBALD

Dunn's (1920) data on the cross, agouti × piebald, indicated a cross-over percentage, between the genes for agouti and piebald, of only 46.23 ± 1.20, in a total of 783 young produced by a back cross of F_1 animals to the double recessive, non-agouti piebald. This shows a deviation of 3.77 per cent. from the normal 50 per cent. value expected if the genes for agouti and piebald assort independently. This deviation is barely more than three times the probable error. The data were also taken incidentally from crosses in which the primary object was to ascertain the relationship between the genes for pink-eye and piebald. This may have influenced the final result in some way, such as less attention being placed on the discrimination of agouti. Therefore further data seemed especially desirable on this relationship.

All later crosses were made with reference only to the agouti and piebald factors. The original matings were all for coupling, the cross being agouti self × non-agouti piebald (AASS × aass). The F_1 animals were heterozygous for the two factors (AaSs). Such animals should form four classes of gametes (1) AS, (2) As, (3) aS, (4) as. If the two genes are independent, these classes should be equal in number; if linked, classes (1) and (4) should be in excess. When such F_1 animals are back crossed to the double recessive, non-agouti piebald, the distribution is obtained which is shown in Table I opposite "new data."

TABLE I

RESULTS OF A BACK CROSS BETWEEN F_1 AGOUTI SELF MICE (FROM AGOUTI SELF, AS, × NON-AGOUTI PIEBALD, as) AND NON-AGOUTI PIEBALD

		AS	As	aS	as	Total	Cross-over Per Cent.
Dunn's data	Obs.	221	179	183	200	783	46.23 ± 1.20
	Exp.	195.75	195.75	195.75	195.75		
New data	Obs.	112	102	110	108	432	49.07 ± 1.61
	Exp.	108	108	108	108		
Combined data	Obs.	333	281	293	308	1,215	47.25 ± 0.96
	Exp.	303.75	303.75	303.75	303.75		

The distribution of the new and more critical data is excellent and excludes any interpretation of linkage between the two genes A and s. The cross-over classes, consisting of agouti piebald and non-agouti self, together number 212. This is 49.07 ± 1.61 per cent. of the total number of young raised, viz., 432, and the deviation is less than one per cent. from the cross-over value expected in independent assortment, which result is well within the probable error, ± 1.61.

Combining Dunn's data with the new data gives a larger number of animals on which to base conclusions. Also, any slight deviation from the normal independent segregation due to random sampling will tend to disappear when larger numbers are involved. On the other hand, a small excess of the non-cross-over class, if it consistently appears in both sets of data, must be considered significant, should it be more than three times the probable error. The combined data are shown in the lower lines of Table I. Here the cross-over classes consist of agouti piebald and non-agouti self, and include 574 individuals. This is 47.25 per cent. of the 1,215 animals raised, and has a probable error of ± 0.96. The deviation of the cross-over value from the 50 per cent. value expected in independent assortment is 2.75 a figure within the range of three times the probable error (± 2.88). The new data and the combined data agree in showing that the two genes are independent.

The Relation of Black-eyed-white Spotting to Agouti

In this section will be presented further data on the relation of black-eyed-white spotting to agouti. It is to be expected that these genes should show no linkage. Little (1912 and 1917) demonstrated the independence of yellow in relation to black-eyed-white. Since yellow, agouti, light-bellied agouti, and non-agouti are multiple allelomorphs, black-eyed-white and agouti should show the same relation to each other as black-eyed-white and yellow. Yet no direct crosses had been made to test the

point prior to the beginning of this work, and the data obtained will, therefore, have value.

Reciprocal matings were made. In the coupling series agouti black-eyed-whites (AAWwss) were crossed with non-agouti piebalds (aawwss). Since both parents were homozygous for piebald (ss), that symbol may be omitted hereafter. The F_1 agouti black-eyed-white young (AaWw) were back-crossed to the double recessive (aaww). The distribution obtained is shown in the coupling series of Table II to the right of "new data." There is an apparent excess of black-eyed-whites and agoutis, perhaps due to random sampling and inexperienced grading of some individuals. But this excess is distributed between both the cross-over and the non-cross-over classes, which consequently are not affected, the numbers being 176, 186. This gives a cross-over percentage of 48.61 ± 1.90.

TABLE II

BACK CROSSES BETWEEN F_1 AGOUTI BLACK-EYED-WHITE MICE (AaWw) AND THE DOUBLE RECESSIVE, NON-AGOUTI PIEBALD (aaww)

Test for	Data		AW	Aw	aW	aw	Total	Cross-over Per Cent.
Coupling . .	Dunn's data	Obs.	36	22	22	34	114	38.59 ± 3.96
		Exp.	28.5	28.5	28.5	28.5		
	New data . . .	Obs.	114	90	86	72	362	48.61 ± 1.76
		Exp.	90.5	90.5	90.5	90.5		
	Combined . .	Obs.	150	112	108	106	476	46.21 ± 1.53
		Exp.	119	119	119	119		
Repulsion .	Dunn's data	Obs.[1]	77	76	76	77	306	50.32 ± 1.92
		Exp.	76.5	76.5	76.5	76.5		
		Obs.	28	27	25	32	112	53.57 ± 3.17
		Exp.	28	28	28	28		
	New data . . .	Obs.	50	69	52	59	230	47.39 ± 2.21
		Exp.	57.5	57.5	57.5	57.5		
	Combined . .	Obs.	155	172	153	168	648	49.84 ± 1.32
		Exp.	162	162	162	162		
			Cross-overs		Non cross-overs			
Coupling and repulsion combined	Dunn's data	Obs.	258		274		532	48.49 ± 1.45
		Exp.	266		266			
	New data . . .	Obs.	285		307		592	48.14 ± 1.38
		Exp.	296		296			
	Combined . .	Obs.	543		581		1124	48.30 ± 1.00
		Exp.	562		562			

[1] The cross-over and non-cross-over classes were given as 154 and 152, respectively.

The deviation from the normal distribution is less than the probable error and therefore not significant.

In the repulsion series the genes for agouti and black-eyed-white entered separately into the F_1 zygote. When back crossed these agouti black-eyed-white young gave the distribution recorded in the repulsion series of Table II opposite "new data." Here the cross-overs number 109, and the non-cross-overs 121, whereas equal numbers are expected. This is a fair distribution for the small numbers raised. Combining the new data on the reciprocal crosses gives 285 cross-overs, or 48.14 per cent. of the 592 young raised, with a probable error of ± 1.38.

Combining Dunn's data with the new data, there are 543 and 581 animals in the cross-over and non-cross-over classes, respectively. The cross-over percentage is 48.30 ± 1.00. The deviation from the normal distribution is less than twice the probable error. The data, separately and combined, clearly show the independence of the two genes.

The Relation of Black-eyed-white Spotting to Pink-Eye

Previous work by Haldane *et al.* has shown pink-eye to be linked with albinism. Detlefsen (1916) described black-eyed-white mice carrying pink-eye, which resembled albinos, but the genes segregated in the second generation. No back-crosses were made in his experiments. No direct intensive crosses hitherto published have dealt with the possible linkage relation between these genes.

In a cross made for such a study, the genes for black-eyed-white spotting and pink-eye entered separately. Accordingly the F_1 gametes should give equal numbers of the parental non-cross-over types (WP and wp), and of the new cross-over combinations (Wp and wP), if the genes W and p are independent. If linked, they should, in the back-cross, show repulsion and the former combinations be in excess of the latter. In Table III opposite "new data" of the repulsion series is shown the dis-

tribution of the young obtained when F_1 black-eyed-whites from the above cross were mated with pink-eyed piebalds, the double recessives. The cross-over classes number 134, or 46.68 ± 1.99 per cent. of the total 281 animals raised. The deviation from the distribution expected is within the probable error and indicates independent segregation.

The reciprocal cross was also made in which the two genes entered together in the same parent. The F_1 gametes should again consist of equal numbers of two classes, WP and wp (the cross-overs), and wP and Wp (the non-cross-overs), if independent segregation occurs. If linkage is shown, the latter class should be larger. The actual distribution is shown in Table III opposite "new data" of the coupling series. The agreement with expectation is poor due to small numbers (77). But since the cross-overs exceed the non-cross-overs, it is clear that no linkage exists. The cross-over per cent. is large (59.74 ± 3.77), but the deviation is still less than three times the probable error. By combining both coupling and repulsion results recorded in the new data, a total of 358 animals is obtained, of which 180 are cross-overs. This gives a cross-over percentage of 50.27 ± 1.78. The deviation from the normal distribution is 0.27 per cent., a figure well within the probable error.

These crosses, as well as Dunn's, show consistently the independence of the black-eyed-white and pink-eye genes. Further evidence may be had by adding together the cross-over and non-cross-over classes of the two sets of data as shown in Table III. The cross-overs number 330 and constitute 51.48 ± 1.32 per cent. of the total 641 animals raised. The deviation from the expected distribution is 1.48 and not significant. The number of young raised does not equal that of other crosses, for the consistency with which these data, in agreement with Detlefsen's observations, show the independence of the two genes, warranted the discontinuance of further breeding.

TABLE III

BACK CROSS BETWEEN F_1 BLACK-EYED-WHITE, HETEROZYGOUS FOR p AND W, AND THE DOUBLE RECESSIVE, PINK-EYED PIEBALD (wwpp)

Series	Data		PW	Pw	pW	pw	Total	Cross-over Pct.
Coupling . .	Dunn's data	Obs.	20	11	9	19	59	66.10 ± 4.14
		Exp.	14.75	14.75	14.75	14.75		
		Obs.	21	17	21	21	80	52.50 ± 3.76
		Exp.	20	20	20	20		
	New data. . .	Obs.	19	20	11	27	77	59.74 ± 3.77
		Exp.	19.25	19.25	19.25	19.25		
	Combined . .	Obs.	60	48	41	67	216	63.42 ± 2.23
		Exp.	54	54	54	54		
Repulsion .	Dunn's data	Obs.	16	21	22	23	82	52.43 ± 3.70
		Exp.	20.5	20.5	20.5	20.5		
		Obs.	18	14	12	18	62	41.90 ± 4.22
		Exp.	15.5	15.5	15.5	15.5		
	New data. . .	Obs.	75	62	72	72	218	47.68 ± 1.99
		Exp.	70.25	70.25	70.25	70.25		
	Combined . .	Obs.	109	97	106	113	425	47.76 ± 1.63
		Exp.	106.25	106.25	106.25	106.25		
Coupling and repulsion			Cross-overs		Non-cross-overs			
	Dunn's data	Obs.	150		133		283	53.04 ± 1.99
		Exp.	141.5		141.5			
	New data. . .	Obs.	180		178		358	50.27 ± 1.78
		Exp.	179		179			
	Combined . .	Obs.	330		311		641	51.48 ± 1.32
		Exp.	320.5		320.5			

SUMMARY

In agreement with the views of previous investigators, it is shown by new and conclusive experimental data that the following pairs of genes in mice assort independently and are not linked. On the chromosome hypothesis, the members of each pair are located in different chromosomes: (1) the genes for agouti (A) and for piebald spotting (s); (2) the genes for agouti (A) and for black-eyed-white spotting (W); (3) the genes for pink-eye (p) and for black-eyed-white spotting (W).

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SHORT EARS AN AUTOSOMAL MUTATION IN THE HOUSE MOUSE[1]

CLARA J. LYNCH

Introduction

Although the house mouse has been one of the favorite mammals used for the collection of Mendelian data, the number of known loci falls far short of the number of chromosomes observed in the germ cells of this species. Therefore, any addition to the list of Mendelian characters in this form should be a matter of interest to the geneticist.

For several years, mice with unusually small ears have been known to exist, but, I believe, no description of the variation has been reported in the literature. The sterility exhibited by many of the individuals used in the following experiments has hindered the collection of data so that the amount is not large, but it has seemed advisable to put on record the results thus far obtained.

The data are based upon observations made at the earliest stage at which it was possible to distinguish with accuracy between the long- and short-eared types. Depletion of litters occurring previous to that time was disregarded.

Description of the Character

The mutation was found in stock which originally came from the Lathrop mouse farm and consists in a noticeable difference in the size of the ear. The pinna is about one half as long as that of the normal ear and usually one or two millimeters less broad but the position in which it is held lying close to the head makes it appear smaller than it actually is. In outline it is less regularly curved than the normal, a flattening near the tip of the ear and one in

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A. Mouse showing the mutation "short ears."
B. An F_1 hybrid from a cross between a short-eared and a long-eared mouse. Ears similar to normal.

the outer margin being fairly constant features. It is usually thick and rather fleshy in appearance. The distribution of hair on the surface of the ear is similar to the normal.

EXPERIMENTAL INVESTIGATION

The Mutant Out-crossed.—The first two experiments showed immediately that the character "short ears" is recessive and that it is not sex-linked. Seven crosses were made between short-eared males and a number of long-eared females which were taken from sources other than the Lathrop stock or from Lathrop strains which

had never been known to produce any short-eared mice. In the F_1 there appeared seventeen young of which ten were males and seven females (Table I). The ears of all

TABLE I

SHORT-EARED MALES CROSSED TO LONG-EARED FEMALES

Mating	F_1 Long	
	♂ ♂	♀ ♀
178	1	1
1369–1	1	1
1369–2	2	0
1369–3	2	0
1443–b	2	2
1443–d1	1	2
1443–d2	1	1
Total	10	7

these mice were long—in fact, indistinguishable from the ears of normal mice. In the reciprocal cross, where the male parent was long-eared and the female short, the offspring were again all long-eared and also comprised members of both sexes. The numbers obtained in the second case (mating 1790, 1 male and 2 females) are very small, partly owing to an unusual amount of destruction of the young which happened to occur in this type of cross, but they indicate that sex-linkage is not involved. Were the gene for short ears located in the sex chromosome, "criss-cross" inheritance would result from this mating and the sons would resemble the female parent and the daughters would be like the male parent since the sons would receive their single X chromosome carrying the short-ear gene from their short-eared mother, while the daughters would receive one X containing the short-ear gene from the mother, and the other X carrying the dominant long-ear gene from the father. This event was not realized—both sexes were of the same type, showing that both kinds of sperm formed by the long-eared father, whether they were male-producing or female-producing, carried the normal allelomorph of the gene for short ears and determined the appearance of the

long-eared F_1 individuals. Since it occurred in both types of sperm the short-ear gene must be located in one of the autosomes.

The Back-cross.—The back-cross between long-eared mice, heterozygous for short ears and short-eared mice, gave 51 long-eared animals to 43 with short ears (the sums of the figures in Tables II and III). On the assumption that the character is due to one gene, the expected back-cross ratio is 1 : 1. In a total of 94, the expectation for the two classes would be 47 : 47. The actual numbers obtained fit the calculated sufficiently well to justify the conclusion that short ears depend upon a single pair of genes.

TABLE II

BACK-CROSS. HETEROZYGOUS F_1 MALES CROSSED TO SHORT-EARED FEMALES

Mating	Long			Short		
	♂♂	♀♀	Sex Not Recorded	♂♂	♀♀	Sex Not Recorded
1612–RF	1	3				
1612–HT				3	1	
1654–a	2	1		2	1	
1654–2			3			
1656–B	3					
1656–1		1		3		
1656–2			1			1
1656–T	1	1				
1656–RH			2			
1656	3	1				
1655						3
	10	7	6	8	2	4
Total	23			14		

In conformity with the results of the first test, the back-cross also shows that there is no sex-linkage concerned in the transmission of the new character. The long-eared F_1 males (obtained by crossing normal females with short-eared males) were bred to short-eared females. If the new gene were sex-linked, the F_1 male would have but one "dose" of the allelomorph long ears. It would be

TABLE III

BACK-CROSS. HETEROZYGOUS F_1 FEMALES CROSSED TO SHORT-EARED MALES

Mating	Long			Short		
	♂♂	♀♀	Sex Not Recorded	♂♂	♀♀	Sex Not Recorded
1655b–1				2	3	
1655a–1	1	2		1	2	
1655b–2		2			1	
1655a–2	1	1		1	3	
1655a1–1	2	3		1		
1655b–3	1	1		2	1	
1655a1–2	1	1		1		
1655			2			3
1719–1	1	3		1	1	1
1719–2	3				1	
1758	1	1	1	1	1	2
	11	14	3	10	13	6
Total	28			29		

carried by the single X chromosome and distributed only to his daughters, which would have long ears. His sons would not receive it; therefore they all would be short-eared like the maternal parent.

The offspring from this cross are listed in Table II. In a few cases the mice escaped or were destroyed by the parents before the sex was recorded. There were, in ad-

TABLE IV

HETEROZYGOUS LONG-EARED MALES BY HETEROZYGOUS LONG-EARED FEMALES

Mating	Long	Short
1655–5	4	1
1723–2	2	4
863	3	
1758–2	2	1
Total	11	6

TABLE V

SHORT-EARED MALES BY SHORT-EARED FEMALES

Mating	Short ♂♂	Short ♀♀
1443–1	4	
1727–1	3	2
1727–2	1	3
Total	8	5

dition, ten males and seven females which had long ears and eight males and two females with short ears. The appearance of individuals of each sex in each class shows that the gene must not be carried by the X chromosome.

The First Filial Generation Inbred.—A very small number of mice were obtained from inbreeding the long-eared heterozygous F_1 offspring. These gave, in the F_2, 11 long to 6 short (Table IV). In a total of 17 individuals on a one-factor basis, the numbers calculated for a 3:1 ratio would be 12.75 to 4.25. The results are consistent with our previous conclusion as to the number of genes involved.

The Inbred Recessive.—The test of the inbred recessive demonstrates that the character breeds true. Three matings between short-eared males and females have yielded 13 young, all with short ears (Table V).

CONCLUSION

The data given above show that the mutation "short ears" which appears as a perfectly definite and easily distinguishable character in mice behaves as a recessive and is dependent upon a single gene which is not sex-linked. Other possible linkage relationships have not yet been worked out.

VARIATION AND HEREDITY IN LUPINUS

DR. LEONAS L. BURLINGAME

STANFORD UNIVERSITY, CAL.

THE great majority of genetic investigations have been carried on with domesticated plants that have been long under cultivation. There are, of course, many entirely good reasons for this fact. It has, however, always seemed to the author that the principles of genetics might, perhaps, be more successfully approached by beginning with wild plants. For this reason he has been bringing into the garden during the past several years a considerable number of wild species which appeared to offer desirable material for experimentation.

His attention was first directed to *Lupinus* through the difficulties that appeared to beset the systematist in arriving at a satisfactory classification of the species. It seemed possible that the difficulty might be due to the fact that the species are of recent origin and, so, very close together. Under the circumstances that a genus is either now or has recently been in a mutable state it would seem likely that natural selection would not yet have had time to weed out all the forms doomed to perish through lack of adaptation or insufficient vigor.

While discussing this question one of his colleagues mentioned the fact that the form recently (1911) described under the name of *Lupinus pipersmithii* Heller (5) grows near and that it sometimes has pink flowers. An investigation during the spring of 1914 not only confirmed this statement, but also showed that pink flowers are not uncommon in two closely related species—*L. vallicola apricus* (Greene) (4) and *L. nanus* Dougl. (7). These three species are all close to one another but apparently satisfactorily separable by the systematist.

This will be clear from the parallel descriptions given below. That each of the species may produce variant types with some characters of the others will appear in our further discussion.

L. pipersmithii Heller	*L. vallicola* (Greene) *apricus*	*L. nanus* Dougl.
Annual	Annual	Annual
Somewhat pubescent below with white hairs, densely so above, especially so in the inflorescences.	Rather shortly and sparsely pilose.	Villous or finely pubescent.
Branches several from the base, 40 cm. high, straw colored, rather prominently ridged.	Branches few to several, almost upright, firm, from near the base, 30 to 45 cm. high.	Slender, not succulent, often branching from near the base, 15 to 37 cm. high.
Petioles slender, about 5 cm. long or uppermost somewhat shorter.		Petioles ½ to 3 times longer than leaflets.
Leaflets about 8.2 cm. long, 2 mm. wide above gradually tapering to the base, apex slightly narrowed and acutish, sparingly appressed pubescent above, more so beneath, light green in color.	Leaflets about 7, oblong-linear, acute, about 1.5 cm. long, thin, sparsely appressed pubescent.	Leaflets 7 or 8, linear to oblanceolate, 1.2 to 2.5 cm. long, usually acute.
Stipules subulate, green, 6–7 mm. long.		
Peduncles 5 cm. long, or less, the inflorescence about the same length.	Racemes sub-sessile, 5 to 8.6 cm. long in full flower.	Raceme loose, short peduncled, 7.5 to 17.5 cm. long.
Flowers in 3 or 4 whorls, the internodes except the uppermost longer than the flowers.	Flowers in 4 or 5 verticils, these rarely indistinct.	Flowers fragrant and in several distinct or somewhat indistinct whorls.
Bracts subulate, 4 mm. long, caducous.		Bracts exceeding the calyx, deciduous.
Pedicels 4 mm. long, slender, villous with short white hairs.		Pedicels about 6 mm. long.
Calyx white villous but the green showing underneath; upper lip long, parted for nearly 3 mm., the lobes lanceolate with a broad V-shaped sinus, the whole broadly ovate when spread out, 4 mm. wide at base; the lower lip oblong, 5 mm. long, 2 mm. wide, 3-toothed at the apex.	Calyx—broad upper lip deeply cleft, the lower entire, the whole exterior appressed villous but not densely so.	Upper lip deeply cleft, lower 3-dentate, the middle tooth sometimes obscure or wanting.

Corolla bright violet blue, 7 mm. long, 5 mm. deep, distance between apices of wings and banner less than 2 mm.	Corolla (blue) broader than long, the breadth about 10 mm.	Corolla blue, 10–12 mm. long.
Banner appearing as if shorter than the wings, the edges turned back and slightly crinkled above, obovate-cuneate when spread out, 6 mm. wide, with a shallow median groove turning purple or violet in age.	Banner with white middle spot, purple dotted along the median sulcus, turning reddish purple in age.	Banner with white middle spot with purple dotted sulcus turning reddish purple in age, orbicular retuse with the sides reflexed.
Wings inflated, open dorsally and at the apex exposing the keel, the ventral edge closed, raised into a low sharp keel, the individual wings obovate-oblong when spread out, 5 mm. wide just above the middle, then obliquely rounded to the broad blunt apex.		Wings lightly joined, forming an obliquely ovate, inflated sac.
Keel glabrous, moderately curved, 2 mm. deep across the middle, the apex purple.	Keel naked (Greene) or bearded on the apical half (Smith).	Keel falcate, ciliate from above the middle.
Pods yellowish, villous, 2.5 cm. long, 4 mm. wide, about 7-seeded.	Pod 2.5 cm. long, appressed villous, 3 to 7-seeded.	
Seeds pale flesh-colored, slightly yellow-brown mottled, 2 mm. long, 1.5 mm. wide.	Seeds not strongly compressed, obliquely round-oval, grayish with a few markings and many small dots (Greene). Seeds well marked with prominent dark brown line on each side and rest of the surface thickly marbled or finely dotted with dark grayish brown. (Smith)	

The above descriptions have been taken from the original sources for *L. pipersmithii* (5) and *L. vallicola apricus* (4) and from Jepson's Flora of Middle Western California for *L. nanus*. The three species are illustrated in the accompanying text-figures.

Field Study of Variation.—Field work can be carried on profitably only in years when lupines are abundant. 1914 was a very favorable year for *L. vallicola apricus* and 1919 and 1920 for *L. nanus*.

In 1914 *apricus* and *pipersmithii* were very numerous in a field near the university and much study was devoted to them. Besides the pink forms already mentioned a considerable number of other color variants were noted and seed collected of some of them. Seeds were collected of both the pink and blue forms of *pipersmithii* and have since been cultivated. Smith (10) had earlier collected seeds of these varieties, but had not been successful in cultivating them. Jepson (7) makes note of the variabil-

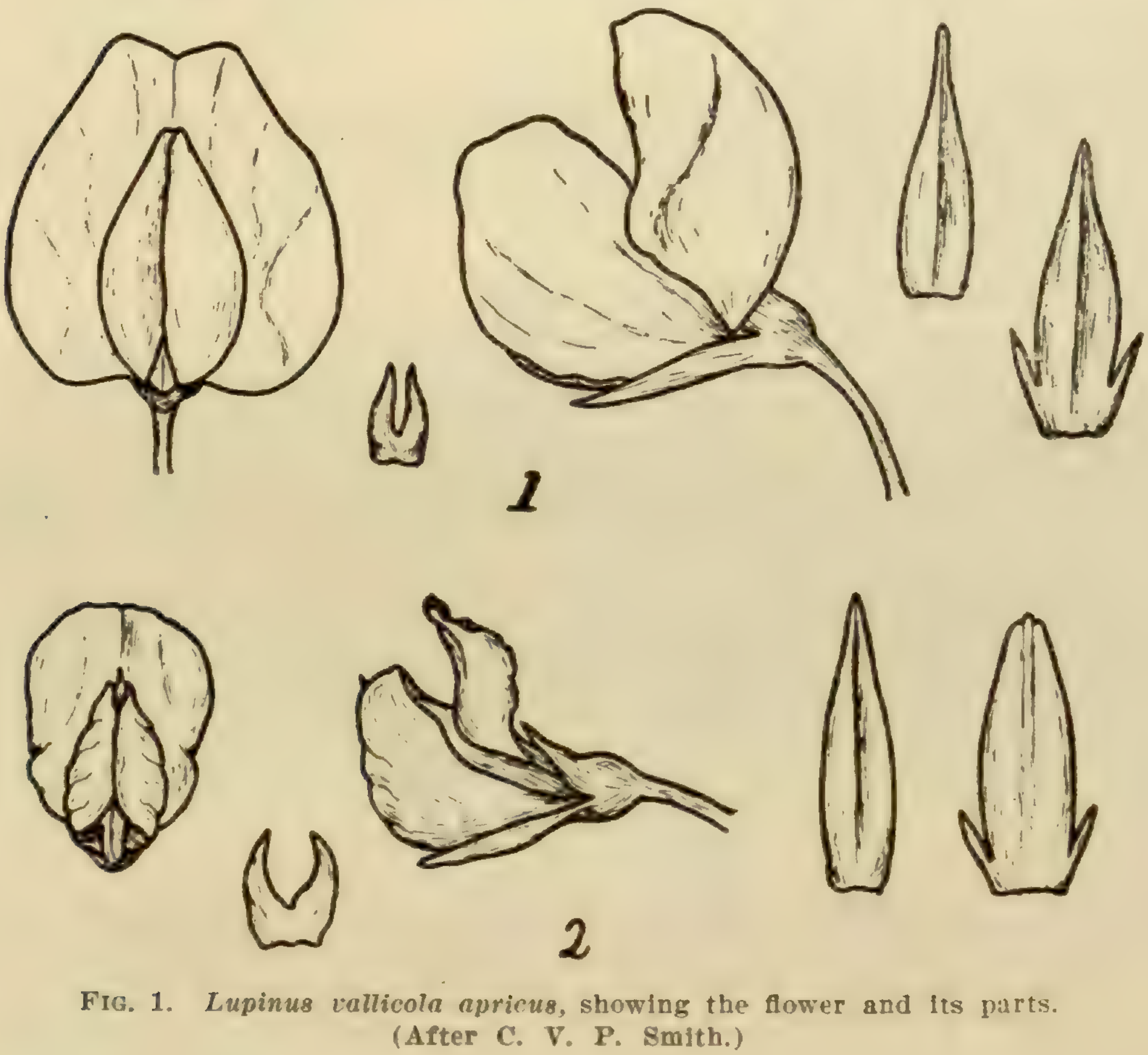

FIG. 1. *Lupinus vallicola apricus*, showing the flower and its parts.
(After C. V. P. Smith.)

ity of *nanus* and Smith mentions variations of size of plant and flower in *apricus*.

The normal color of *apricus* is dark blue and white. After some study it became very obvious that, mixed with the usual types, there were occasional plants with a decidedly lighter blue. One variation consists in a mere lightening of the blue. This type was called light blue

and plants were bagged for seed collection. Another type resembled this in color except that both banner and wings were distinctly striped. The amount of blue and white varies somewhat but the type is always recognizable. In some cases one might correctly speak of a light blue flower

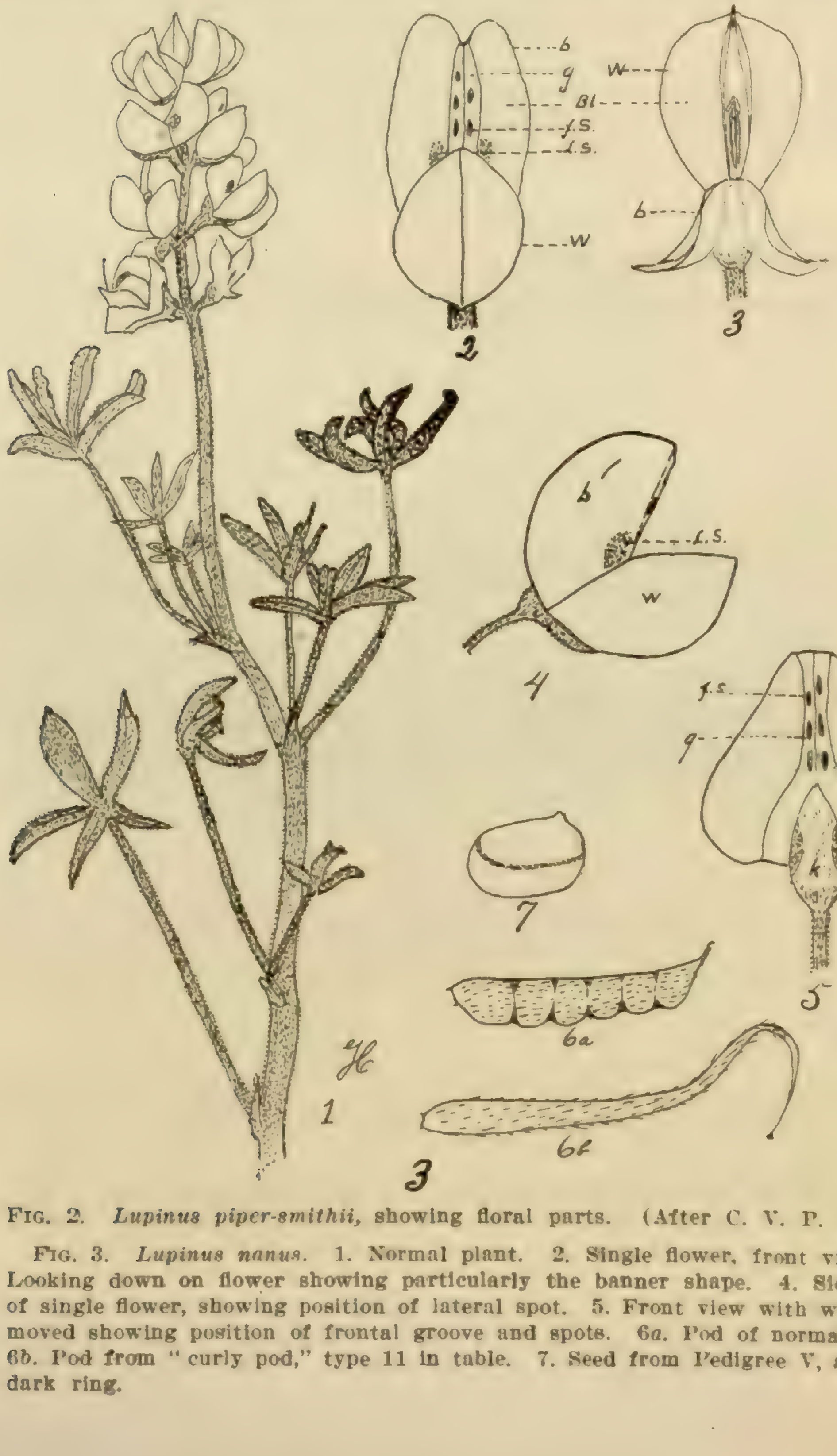

FIG. 2. *Lupinus piper-smithii,* showing floral parts. (After C. V. P. Smith.)

FIG. 3. *Lupinus nanus.* 1. Normal plant. 2. Single flower, front view. 3. Looking down on flower showing particularly the banner shape. 4. Side view of single flower, showing position of lateral spot. 5. Front view with wings removed showing position of frontal groove and spots. 6*a*. Pod of normal plant. 6*b*. Pod from "curly pod," type 11 in table. 7. Seed from Pedigree V, showing dark ring.

with white stripes or veining. In other, and rarer, cases it is apparently a white flower veined with blue. Seeds of this type were collected and have since been grown under the name of striped white. This first season it was not realized that the two lighter forms were distinct. Fortunately the seeds of each individual plant were saved and grown separately, so that the distinction became evident in 1917, the first year in which successful cultures were grown.

Pink *apricus* were also fairly common that year and have been occasionally noted since. Seeds were saved and have since been grown.

Two white forms were observed that year. One appeared to be plain white. This type has been frequently observed since in this and other species. A single plant was also observed in which the color was a cream or yellowish white. Seeds of both sorts of plants were saved but have so far failed to grow.

A few scattering observations were made on *nanus* from year to year, but it was not until 1917 that a field was found in which variations were numerous and conspicuous. Pinks, light blues, striped whites, and whites were all abundant in a field near the town of Woodside. Specimens of all these types were staked out for seed but unfortunately attracted the attention of the small boy and no seeds were secured. In 1919 3 fields were found some 3 miles west of the university in which numerous variations were in full bloom. A careful study was made and an attempt made to discover and describe every variation. Twenty-seven plants were labeled and described for seed collection. They represented 12 separate and distinct variations or combinations. All the variations but two are concerned with flower color. In the description of *nanus* it will be noted that the normal plant has a blue and white corolla having a reflexed banner with a white front down the middle of which there is a groove marked with a number of small dark spots. In the variants the normal dark blue of the banner and

wings may be replaced by the light blue or striped white described for *apricus* or the whole corolla may be white or pink and white.

All the dark blue, light blue and striped white plants had a white front on the banner with blue, blue-green, or no spots in the groove. White corollas had either white or lemon-yellow fronts with or without the dark spots. Normal dark blue plants have the color uniform on banner and wings or else with a deeper spot at the angle where the reflexed banner joins the wings. In light blues this spot may be dark blue, light blue, greenish blue or absent altogether. Whites have this spot faintly blue, bright orange, or absent. The orange spots may occur with either white or lemon-yellow fronts.

A reddish-purple corolla was found on a number of plants which were distinguished by unusually large and fine flower clusters. In some of them the flowers had fallen and pods formed on some earlier branches. The pods in every case were of a very peculiar type. Instead of the elongated, nearly straight and somewhat flattened and constricted type commonly found these plants had pods long, tapering, nearly round, and frequently much coiled. This was at first supposed to be due to attacks of insects or fungi. Diligent search failed, however, to show any such infection or infestation. And when it was later discovered that the plants which would produce these curly pods could be identified in the early flowering stage it was realized that this whole group of characters constituted an interesting variation. A number of plants were labelled and some bagged for seed. Although many seed capsules were examined, no good seed were secured. It appears highly probable that this form is completely sterile, though there is a chance that the failure to set seed may have been due to unfavorable weather. In 1920 neither this nor any of the other variations were to be found in that same field. Diligent search, in fact, has failed to discover it anywhere. It is much to be regretted that good seed of this

form can not be secured, inasmuch as the somatic variations involved appear to be more profound and complicated than in any of those so far studied.

The only other form variations, aside from mere size, so far observed are two modifications of the banner. In one plant of *nanus* in 1919 the banner was hooded in the fashion of some varieties of sweet peas. This same variation had been previously noted in *apricus* in 1914. *Apricus* also occasionally yields plants with the abbreviated banner characteristic of *pipersmithii*. So far seeds of neither of these variations have been collected, though attempts have been made.

The table below summarizes the variations observed in 1919. This season's study yielded no new types and failed of some of those found the year before.

Number	Color and Markings			Front	Lateral	Other Characters
	Corolla	Veining	Front	Spots	Spots	
1.....	Light blue	Veined	White	Dark blue	.....	.....
2.....	"	.....	"	"	Dark blue	Dwarf
3.....	"	.....	"	"	Faint blue	.....
4.....	"	.....	"	Greenish	Greenish	.....
5.....	White	.....	Lemon-yellow	.....	Faint blue	.....
6.....	"	.....	Lemon-yellow	.....	.....	.....
7.....	"	.....	White	Dark blue	Orange	.....
8.....	"	.....	Lemon-yellow	.....	"	.....
9.....	Pink	.....	White	.....	Faint blue	Dwarf size
10.....	"	.....	"	Dark blue	Blue	.....
11.....	Reddish purple	.....	"	"	.....	Curly pods
12.....	Reddish purple	.....	"	"	Blue	Hooded banner

The variations in size of all three species are very considerable, no doubt due in large part in the field to differences of soil, moisture, shade and exposure. When grown in the garden side by side *nanus* is the largest and most vigorous and *pipersmithii* the smallest. In the field *nanus* is often smaller than *apricus*, though the flower cluster is usually much larger. This difference is possibly due to the fact that it grows in more open, ex-

posed places and usually in poorer soil. No cultures of different-sized plants of the same species have yet been grown and critically compared. Some casual observations, however, indicate that there are probably heritable size differences in *apricus*.

Reference to the specific descriptions will show that some taxonomists have been inclined to base classification in part on the color, shape and markings of the seeds. Our observations would indicate that this is not a very safe criterion, particularly in reference to color and markings. Seeds of *nanus* are the largest. They could usually be picked out of a mixture with *apricus* by size and shape, but not by the color or markings. No character is more subject to variation than this. This does not mean that the character is a fluctuating one, but that there are a great many differently colored and marked seed varieties. All the seeds of any one plant are alike (with certain exceptions to be noted in a later paragraph), as one would anticipate since the color and markings are seed-coat characters and hence genetically all alike on any particular plant.

Dark blue *nanus* and *apricus* plants have dark seeds, whites and pinks have light seeds, light blues and striped whites have seeds of intermediate color. Pinks and light blues have a yellowish-brown tone and are flattened and without other markings. Striped whites are longer and thicker in proportion to the width, of either a bluish or yellowish tone with a conspicuous crooked line on either side forming a continuous ring about the shorter perimeter of the seed. This ring does not occur on the seeds of pinks or whites. It is present on some races of dark blues and absent on others. The statements just made apply particularly to those races which have been cultivated for some time in the experiment garden. Field observation confirms them in part, but reveals a considerable number of other variations in color and marking which have not yet been cultivated and about the genetic behavior of which nothing is yet known. The only case

so far noted in which the seeds of a single plant of *apricus* are not entirely uniform is that of two tones in a striped white variety. An attempt has been made to cultivate the two sorts separately and to determine the ratio in which they occur. Cultures have not yet proved successful. In some collections of seed the ratio approximates a 3:1, but in others it is nearer 8:1, but whether any significance is to be attached to these results has not yet been definitely determined.

It is a curious fact that, although lupines yield an enormous number of seeds and the plants often literally cover acres of space they are nevertheless very capricious in their occurrence. Some illustrations of this may be taken at random from our notes. The field near the university where the *apricus* mutants and the pink *piper-smithii* were collected in 1914 has been under observation every year since and has not at any time had many plants or produced a second display of these mutants. A number of pink forms of both species were staked for seed that first year and the stakes left in the ground to mark the site, in the expectation that the same forms would reappear the following year. In no single case were pink flowers found at any of these stations, although they were found the next year at other locations in the same field. The field near Woodside studied in 1917 has been visited each year since. Not only have no mutant forms been found there, but there have been exceedingly few normal ones. The field from which the notes were made for the table on a preceding page was very thoroughly searched again this spring. There were a few dark blue plants, but not a single one of the types which were more or less abundant there last year. These vagaries of distribution doubtless depend in some manner not yet clear on the difficulty of germinating the seeds.

Pollination and Seed Collection.—In 1914 when these observations were begun it was assumed that the lupines were probably frequently cross-pollinated, inasmuch as they appeared to be freely visited by bees. It was a

matter of some surprise to find that the forms of *apricus* and *pipersmithii* which were brought into cultivation did not indicate this to be true. The pinks bred true in both species. Experiments to determine self-pollination by bagging or screening the plants with fine-meshed wire cages showed no diminution in the harvest. Furthermore, in the following years different strains grown in adjacent rows showed no sign of crossing. It was then assumed that the same would be true of *nanus* and flower clusters were bagged in the field for seeds—not to insure selfing, but merely to prevent the seeds from being scattered by the explosive dehiscence of the pods. The results were wholly negative, resulting in a failure to secure any seed that year. The appearance of the contents of the paper bags first used led to the supposition that possibly the failure to set seed was due to the bags. Careful experiments were therefore made the following season by inclosing whole plants in cages of fine screen wire or cheesecloth. In no case did this result in setting seed. It appears, therefore, that *nanus* is dependent on bees for pollination. On the other hand, cultures derived from white-flowered *nanus* have shown that the bees act in part merely as a mechanical agent, for part of the progeny was white and part blue. These results agree well with and serve to explain the greater number of variations found in *nanus*.

Pollen Sterility.—It has been maintained by a number of authors at one time or another that variability in nature is very largely a matter of hybridity and that sterile pollen is a more or less certain indication of the hybrid nature of a species (6). Having found two closely related species both variable and in a closely similar manner, it became a matter of interest to study the comparative sterility of close-pollinated and cross-pollinated species. In order to determine this matter a large number of plants belonging to all the varieties in cultivation at the time in the garden were examined. Mounts of pollen from three different flowers from each plant were

made. Each slide was so prepared that about 100 pollen grains would be visible in a single field of the microscope. All the grains in the field were then counted and the percentage of sterile grains calculated and averaged. Only two plants showed more than 3½ per cent. of sterile pollen. One normal dark blue plant showed 40 per cent., 39 per cent. and 0 per cent. in three flowers. One striped white showed 9 per cent., 6 per cent. and 0 per cent. in three counts. One hybrid light blue, one dark blue and one pink showed no infertile pollen. The one plant with a high degree of sterility was a selfed dark blue *apricus*. Only a few plants of *namus* were available, but they showed no poorer pollen than *apricus* and *pipersmithii*.

Seed Germination.—In a previous paragraph it has been pointed out that, although lupines produce immense quantities of seeds, field germination is apparently poor—at least an abundant seed harvest is likely to be followed by a poor stand of plants the following season. Our earlier attempts to grow them in the garden were practically a total failure.

The first attempt to grow controlled cultures was made in the winter and spring of 1914–'15. Four hundred seeds were planted in pots in ordinary unsterilized garden soil and 400 more in pots of sterilized soil (sterilized in an Arnold steam sterilizer). The seeds themselves were planted dry without treatment of any sort. The results were almost a total failure. About 5 per cent. of the seeds produced seedlings. Of these all but 5 or 6 were killed as seedlings by the attacks of soil fungi or just simply died. Two plants lived to flower, but failed to set seed. It appeared evident that greenhouse cultures under the conditions then available were likely to be unprofitable and the remainder of the original seed collections were held over until an experiment garden could be secured.

In 1917 the remaining seeds were planted without treatment in the open garden. Nine plants in all lived to mature seed—one light blue, one striped white, one pink

pipersmithii, three pink *apricus*, and three dark blues. The seeds since used have all been derived from these nine plants, each of which has been assigned a pedigree number.

In 1918 seeds of each of the 1917 plants were again planted. Although about 400 seeds of each were planted in each culture, two pedigrees failed to yield a single plant that year, though a few plants came up the following spring. Pedigree VIII, Light Blue, yielded a culture of 85 plants and pedigree V, Striped White, 28 plants. Thus the highest per cent. of germination did not exceed about 20 per cent., ranging from that down to zero.

In 1919 a series of germination tests were carried out. This had not been possible before on account of the small number of seeds available. A considerable variety of methods were employed. Soaking the seeds in tap and distilled water for weeks is of little value, owing to the failure of the seed to imbibe water. Breaking or cutting the seed coat brings about prompt imbibition of water and consequent swelling, but does not produce a high percentage of germination. Seeds were soaked in water under air pressures up to 140 pounds to the square inch with no noticeable effect.

Since the seeds are small cutting or filing the seed coats is a very arduous affair where cultures of any size are to be grown. Attempts were, therefore, made to find some other means of bringing about the same result. The seed coat appears to be difficult to wet and this was thought to be due to the presence of some oily or waxy constituent. Attempts to dissolve this by means of KOH, various percentages of alcohol, ether, etc., proved entirely unsuccessful.

Soaking in concentrated H_2SO_4 proved the most efficacious method tried. This was applied in parallel series of seeds from the same plant for periods from 5 minutes up to 2 hours. The shorter treatments seemed to produce no effect at all. The longer periods of two hours or over killed the embryos. After some experimentation it

was found that a treatment of one and one half hours would sufficiently char the seed coat to secure practically 100 per cent. of swelling (8). At first it seemed impossible to say whether this might not also injure the embryos. Later it was discovered that whenever any acid penetrated the cotyledons of dark blue plants they turned pink. This color is probably due to the presence of a chromogen similar to or identical with the one which eventually produces the pink or blue flower pigments. Since this color reaction is brought about by even faintly acid solutions, it was thought that it would serve as an effective check against overtreatment with the acid.

After the preliminary experiments had shown the sulphuric acid method to be the best at our disposal a complete series of tests were run on each of the 70 pedigrees available for planting at that time. The dry seeds were placed in the concentrated commercial acid and left for 90 minutes. They were then washed rapidly through several changes of sterile water until the water failed to affect litmus paper after the seeds had stood in it for 20 to 30 minutes. It was found necessary to carry out the washing rapidly since the weak acid readily penetrated into the cotyledons.

After washing the seeds were subject to one of three treatments. Some were left in sterile water until sprouted. Others were removed as soon as they had swelled. The great majority of the pedigrees swelled within 18 hours. A few were completely swelled within 4 hours. In most cases they were left about 18 hours. A third method was to transfer the seeds as soon as washed to a nutrient solution. This did not show any advantage over plain sterilized water. The seeds which were removed from the water after washing and swelling were placed on or between moist blotting papers. It was soon found that those left in the water or placed between papers kept wet and soggy excelled those which were placed on papers merely kept moistened. Twenty-one lots of seed failed to sprout at all, although the percent-

age of swelling was 95 per cent. or better except in two cases, each of which had only 40 per cent. of the seeds swelled. The remaining lots averaged 51 per cent. of sprouted seeds. Two gave 100 per cent., one gave 95 per cent. and 14 were below 25 per cent.

From these results it appears that many seeds which show no observable defects are nevertheless either dead or in a state of dormancy not readily overcome. That the latter is probably the true explanation is indicated by the fact that more seeds treated in this manner actually sprout than when the seed coats are mechanically ruptured. The treatment with the acid seems to act in some manner, possibly by dehydration, as a slight stimulant to sprouting. It is not certain, however, that a larger percentage of viable seedlings is actually produced. Many seeds put forth the radicle in an apparently normal manner, but do not continue growth. Others die at later seedling stages apparently from internal causes, for they have not had opportunity of infection and are growing under the same conditions as others in the same culture.

This spring and winter a number of cultures were tried in which the seed were treated with acid, washed, and then planted directly in the soil out of doors. In every case a good stand was secured averaging about 50 per cent. of the seeds planted. The seedlings of the preceding season were planted in pots and kept in the greenhouse until a vigorous young plant was secured, and then transplanted to the garden. They did very poorly after being transplanted and produced practically no seed. It is uncertain whether the failure was due to faulty technique in transplanting or to the failure of the plant to adapt itself to the change of environment. It is not unlikely that both causes had something to do with the matter. They had been grown in 5-in. pots and transplanted with the whole mass of dirt, but even in that way some disturbance of the root system was unavoidable. In addition to this there was a very hot, dry wind lasting three days about flowering time. This seemed to do a lot of

damage to plants in the field and was no doubt highly injurious to those in the garden as well. It is planned to repeat the experiment again, using paper pots which may be set in the garden without disturbing the roots at all and without interfering with subsequent growth. (These pots have no bottoms.)

Garden Cultures have now been carried through four seasons in some lines and through two or three in the others. Pink *apricus* and *pipersmithii* have proved entirely constant, with one exception. Dark blue *apricus* also breeds true.

The striped whites of Pedigree V for three seasons produced both striped whites and dark blues. In 1918 the culture produced 28 plants which flowered. There were 20 striped whites and 8 dark blues. In 1919 the cultures were so badly injured by transplanting and unfavorable weather that little reliance could be placed on numbers. Some cultures, however, did produce white plants. In the larger, but still unsatisfactory, ones this spring whites have again been produced. Although the numbers are very small, the fact that striped whites give rise to dark blues, striped whites, and some whites is significant. Two cultures of 100 seeds each this spring produced two white plants each and no other sorts. From these data, unsatisfactory as they are, it is probable that striped whites are hybrids between white and dark blue and that white differs from dark blue by a single factor.

The light blues of Pedigree VIII have also been grown through four seasons. In 1918 the original plant produced a progeny of 85 plants, of which 20 were dark blue, 60 light blue, and 5 failed to flower. They have never produced any whites. This season 8 cultures out of 27 produced only light-blue plants. In most cases the numbers were small, but one culture of 200 seeds produced 32 light blues and one plant which did not flower. Three others respectively produced 22 light blues out of 200 seeds, 18 light blues out of 170 seeds, and 9 light

blues out of 150 seeds. Taking into account that four of the eight cultures had only one or two plants and so might have produced dark blues also, this 8 : 27 is probably as close an approximation to a 3 : 1 ratio as could be expected. The data now available would appear to justify the conclusion that the light-blue color is due to a single factor difference and that heterozygotes and homozygotes are phenotypically indistinguishable.

Owing to the fact that a single cross yields only five or six seeds, it has not seemed profitable to attempt hybridizing the light blues and striped whites either with one another or with other forms, until a techinque has been perfected that insures a higher percentage of germination. In *nanus*, which is naturally crossed, certain observations have been made which indicate something of the relations of certain factors to one another. Seeds of a white-flowered plant collected last year from unprotected flowers were grown this year and produced both whites and blues apparently like normal wild ones. This would indicate that the mother plant had been visited by a bee which had effected pollination in part with its own pollen and in part with that of a neighboring dark blue. If this be the true explanation this white was a recessive one. It might have been a heterozygous dominant white, of course, which would be in agreement with the nature of the whites in *apricus*, Pedigree V. Seeds of pink *nanus* collected last year yielded only a half dozen plants, all dark blue. This would point to the conclusion that pink is also recessive to blue. However, these results are too meager to have more than a suggestive value.

Seed characters have also proved constant in inheritance. Dark-blue *apricus* plants invariably have dark seeds, but the particular type of marking differs according to the origin. It is suspected that the two types of seeds found in Pedigree V, striped whites, indicate a genetic difference, possibly distinguish striped whites

from pure whites, but the facts now known do not suffice to prove this.

Mutations have apparently occurred in culture in respect to both flower color and seed-coat markings.

This spring (1920) a single dark blue appeared in a culture of pink *apricus* which had bred true through the three preceding generations. In 1918 a pink arose in a culture of seeds from a wild dark-blue *nanus*. If the relations between pink and dark blue are the same in the two species one of these cases must be a mutation. Hybridization is exceedingly improbable in the case of the pink *apricus*. It is not likely that the dark-blue *nanus* was a hybrid either since it was not collected from a location where this cross would have been likely to occur and only one plant out of a large culture was pink.

In 1918 plant VIII–27 with dark-blue flowers produced seed of the light color characteristic of light blues. These seeds this year produced both light blues and dark blues. In collecting these seeds it was necessary to read the label on the plant and that on the seed box. It is very unlikely that they failed to tally with each other or with the color of the flowers still in bloom on the plant. Several collections were made over a period of two or three weeks and the plant label put in the box at the final collection. Owing to the fact that the seed pods were not opened until after the collections had been finished, no suspicion of anything unusual was entertained until after any sort of check was no longer possible. A mistake might have been made several times in succession, but this is certainly very improbable. The seeds this year are of the usual type. Three light-blue plants produced light seeds and two dark blues produced no seeds.

If this is not a case of mistake in records it is very difficult to offer any explanation of it. Since plant VIII was a hybrid and the factor for dark-blue flower color is linked with that for dark seeds, plant VIII–27 could have arisen either through a mutation in the recessive factor for coat color or by a cross-over of its dominant

allelomorph, so that dark flowers would then be linked with light seeds. In either event plant VIII–27 would be homozygous for dark flowers. It might have been either homozygous or heterozygous for seed-coat color. In the one case it would yield a progeny with dark-blue flowers and light seed coats. In the other all plants would have dark-blue flowers, but there would be three light seed coats to one dark one. The results, however, are both light-blue and dark-blue flowers.

Pedigree VII is dark blue and has bred true for three seasons. This spring culture VII–3–1 produced a single white plant. It was the only plant from 77 seeds. The parent plant was exceptional in that its seeds were lighter in color than usual.

Discussion.—It is realized that the facts presented in the preceding pages are regrettably incomplete. It is hoped, however, that they are sufficient to interest others in lupines as suitable materials for genetic investigation.

The striking parallelism in the mutations occurring in the two species, *apricus* and *nanus*, is certainly a significant phenomenon. All recent work with both plants and animals proves that in varietal crosses homologous chromosomes are freely interchangeable and that allelomorphic factors occupy identical loci in their respective chromosomes. The work with multiple allelomorphic systems clearly indicates that a particular factor may undergo a number of different changes. In *L. apricus* the evidence at hand likewise indicates that the factors producing striped white and light blue respectively are each allelomorphic to that for dark-blue flower color. Whether they are allelomorphic to one another remains to be shown, though that would be a probable supposition.

From the data presented in this paper it can not be said whether flower color and seed-coat color are both due to the same factor or to linked factors. The latter is, however, indicated by two facts. In the first place we already know more heritable patterns of coat color than there are flower colors associated with them. At least

three seed patterns are found in association with dark-blue flowers in different pedigrees. In the second place there is the case of the dark-blue plant, VIII–27, which produced seeds with the light color characteristic of light blues.

In the great majority of mutations described up to the present time the new character is recessive to the normal one (1). They are apparently due to the loss or inhibition of a previously existing factor. It is interesting to note that the two factors for light blue and striped white here reported are both dominant, the former completely and the latter incompletely so. Furthermore, many of the other variations observed are in the nature of additions. Yellow color on the front of the banner and orange lateral spots, although their heredity is not yet known, are certainly to be considered as in the nature of additions.

The mutations in *Lupinus* represent three categories (11). In the whites a positive character has been lost. In the light blues and striped whites a character has been replaced by another. This would naturally be supposed to be due to some alteration of the factor governing the somatic character. In the case of the orange spots and lemon-yellow fronts one is led to suppose the addition of a new factor, especially so in view of the fact that they are not constantly associated with one another or with white flowers. White flowers may occur without either lemon fronts or orange spots or with both or with one and not the other.

These three categories of characters naturally suggest that there are also three sorts of factorial bases for them. Loss of a character appears readily explicable either on the assumption of an actual loss of the factor in the chromosome or of its becoming latent. Multiple allelomorphic factors seem to be located according to the work of Morgan and his associates (8) at identically the same loci in their respective chromosomes and must therefore be thought of as different changes of the same factor.

Additional characters when inseparably linked with an old one might be due to a change in the original factor, but when not linked, or so loosely so as not to maintain a constant association, they would have to be considered as due to a new factor. A new factor might originate by the subdivision of an old one and the subsequent differentiation of one part. In this case as well as in the case of the actual loss of a gene, homologous chromosomes of the hybrid between the new and old form would present the situation originally conceived in the Presence and Absence hypothesis (2) of an actual gene paired with its absence. In the other cases of modified factors this would not be true.

Summary.—1. The genus *Lupinus* presents an assemblage of closely related and difficultly separable species.

2. The present paper reports some results of a 6-year field and garden study of *L. apricus vallicola*, *L. piper-smithii*, and *L. nanus*.

3. The variations described concern the form and color of the flower, the shape and size of the pod, and the color and markings of the seeds.

4. Dark-blue and pink-flowered races breed true.

5. Striped-white flowered races are heterozygous for a single factor, which in the homozygous condition produces white flowers.

6. Light-blue flowers are due to a single dominant factor, indistinguishable in the homozygous and heterozygous condition.

7. Dark seed coats are linked with dark-blue flower color, but probably due to separate factors.

8. The factors for light-blue and striped-white flowers are both allelomorphic to that for dark-blue and not improbably constitute a system of multiple allelomorphs.

9. Mutations are frequent, some are already known to be dominant, and others appear to be in the nature of additions of new characters and factors and so progressive in the sense of de Vries.

10. On account of the frequency of dominant and progressive mutations and notwithstanding the difficulties of seed germination this genus merits the attention of geneticists.

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STUDIES ON PARASITIC COPEPODS OF THE GENUS SALMINCOLA[1]

NATHAN FASTEN

Oregon Agricultural College, Corvallis, Oregon

The parasitic copepods afford a very interesting group of animals for the biologist. Not only do these organisms offer many fascinating problems for the pure scientist, but from the commercial standpoint they are extremely important as parasites of our food and game fishes. There is every degree of parasitism amongst these crustacea, from those which spend a very small portion of their existence as parasites to those which are parasitic throughout almost their entire life. My own studies, covering a period of nearly ten years, have been confined to some of the most highly specialized members of the latter group, belonging to the genus *Salmincola* of the family *Lernæopodidæ.*

The *Salmincola* are parasitic on the *Salmonidæ,* which include such important fishes as salmon, trout, lake herring and whitefish. These copepods are all built on a similar plan and can be easily recognized. The adult females are the ones which are usually encountered, and these may be attached to the delicate membranes of the gills, gill chambers, fins and mouth of the host. Here they hang on and are supplied with a constant stream of fresh blood, which serves as their sole food.

These adult copepods can be readily seen with the naked eye. They are quite large, measuring a few millimeters in length, and are yellowish-white in color. Anteriorly they are fastened to the host by means of two second maxillæ and a chitinized bulla. This last named structure is imbedded in the tissues of the host. Posteriorly each female possesses a pair of slender freely

[1] Delivered before the Biological Club, Oregon Agricultural College.

dangling egg-sacs within which the embryos undergo complete development.

During the last few years our knowledge of the *Salmincola* has been increased largely through the efforts of Wilson and the present writer. Wilson, in 1915,[2] published a key to the various species of *Salmincola* found in North America, while the present writer (1912–1919)[3] has published a series of papers on the behavior, morphology, life-history and economic importance of two of these forms, namely *Salmincola edwardsii* (Olsson) Wilson, which parasitizes the brook trout of our middle-western and eastern states, and *Salmincola falculata* Wilson, which is parasitic on salmon and trout of our Pacific states. In speaking of the former species, Smallwood, in a recent paper,[4] says:

> These parasites are widespread in the United States in the native trout streams, and in Canada and Europe. The first scientific record of this particular parasite is by Linnæus in 1761. It seems strange that an animal could be known for so long and its habits not be understood until within the past five years.

The writer is at present engaged on other species of *Salmincola* which dwell on various salmon and trout of the northwest section of the United States. From all appearances the different stages in the life histories of the various species of *Salmincola* seem to be more or less similar and, therefore, will be briefly outlined.

As already mentioned above, the young larval copepods undergo development within the egg-sacs of the attached females. When these larvæ are mature, they rupture the egg-sacs and escape into the water as minute, freely-swimming organisms that closely resemble free-living pelagic copepods. Although they measure about one thirty-fifth of an inch in length, they are very active and

[2] *Proc. U. S. Nat. Mus.*, Vol. 47, pp. 565–729.

[3] *Report Wis. Fish. Com.*, 1911–12, pp. 12–22. *Jour. An. Beh.*, Vol. 3, pp. 36–60. *Biol. Bull.*, Vol. 27, pp. 115–127. *Biol. Bull.*, Vol. 31, pp. 407–419. *Pub. Puget Sound Biol. Sta.*, Vol. 2, pp. 73–77. *Pub. Puget Sound Biol. Sta.*, Vol. 2, pp. 153–181.

[4] AMER. NAT., Vol. 52, pp. 322–352.

swim about with a snappy spiral dart. They may thus swim about for nearly two days, constantly searching for a host to which to attach themselves. They dart here, there and everywhere: if not successful in meeting a host, they soon die, but if one is found they attach themselves and carry on their life-cycles to completion.

In *Salmincola edwardsii* it has been found that the larval copepods swim about near the surface of the water throughout the day, but at night they sink down to lower depths near the bottom of the stream. These migrations, although contrary to the general migrations of free-living copepods, are, nevertheless, of great benefit to these parasitic forms in that they are parallel with the migrations of the hosts. Brook trout generally feed near the upper surfaces of the streams during the day and at night they sink down to lower levels. This similar behavior on the part of the parasite and the host makes it much easier for the parasite to meet its host and thereby carry out its life-cycle.

The manner in which the larval copepod attaches itself to the host is extremely interesting. Each larva possesses powerful mouth parts and a peculiar attachment filament which aid in the attachment of the organism. On coming in contact with a desirable portion of the host, the parasite first rasps a hole in the tissues by means of its mouth parts. Then the attachment filament is brought in contact with this cavity and by means of the contraction of numerous thin head muscles which are attached to the proximal end of the attachment filament, the bulb-like distal end of the filament is driven into the cavity. The glue-like secretion of the attachment filament as well as the regenerating tissue of the host soon attach the copepod quite securely.

The copepod now undergoes degeneration. It loses its segmentation as well as its plumose swimming feet. The abdomen rounds out, becomes larger and more bag-like in outline. The mouth parts also change their appearance. The mouth itself grows into a prominent tube-like

piercing organ, which is capable of puncturing the tissues of the host for purposes of sucking blood. This is the exact method by means of which the attached parasite feeds itself.

About a week after attachment to the host, the modification of the parasite has been so complete that one can hardly recognize any resemblance between it and the free-living larva from which it was derived. In another week and a half, that is, about two and a half weeks after attachment, the copepods have reached sexual maturity and are ready to undergo fertilization. The males can now be easily distinguished from the females. This was not possible previously. The females are veritable giants as compared with the males, being about three or four times the size of the latter.

The only male ever discovered in the genus *Salmincola* is that of *Salmincola edwardsii*, which has been described and figured in the *Biological Bulletin* for 1914. The writer has just completed the study of another male of a different species of *Salmincola*, namely, *Salmincola beani* Wilson which he recently discovered on the gills of the chinook salmon.[5] This new male shows the same size difference when compared with the female as does the male first mentioned.

Prior to fertilization, the males and females hang side by side on the tissues of the host. In order to accomplish fertilization, the male undergoes a rather peculiar behavior. He begins circling movements and somewhere in his vicinity he comes in contact with a female. As soon as this occurs, the male clasps the female with his maxillipeds and at the same time he releases his hold on the tissues of the host. The male then creeps towards the posterior region of the female's body, in the neighborhood of the genital pores, and here he attaches himself in position for fertilization. The male next bends his abdomen upward toward the genital openings and soon extrudes two pear-shaped pouches known as spermato-

[5] In press, *Biol. Bull.*

phores. These are manipulated by the free maxillæ of the male and are ultimately attached near the genital pores of the female. The spermatophores contain a cement-like material which aids in their attachment. They are also filled with large numbers of mature spermatozoa which wander through the genital pores and become stored within the spermatheca of the female. Here these male gametes remain dormant until the ova of the female are ripe for fertilization. When the wandering of all the spermatozoa has been completed the spermatophores collapse and soon come to resemble transparent, shell-like, yellowish spheres. The female may be fertilized more than once. Oftentimes as many as six spermatophores may be found clinging to the genital pores of some of the females, showing that these have been fertilized three times.

After fertilization, the male drops off the body of the female and soon dies. The female, however, lives on and completes the life-cycle. She now undergoes extreme degeneration, increases enormously in size, and develops a large number of eggs which become clearly visible within her abdomen. At the same time two slender membranous egg-sacs make their appearance at the posterior margin of the female's body. When the ova are ripe they are passed down through the oviducts and as they migrate past the spermatheca they are fertilized by the stored spermatozoa. The embryos are then transferred to the egg-sacs where they carry on their complete development. In about a month the young are liberated as free-swimming larvæ ready to begin the cycle again. In *Salmincola edwardsii* two batches of young are produced, each numbering about one hundred and twenty individuals. After all the young have been liberated, the adult females die and soon deteriorate on the tissues of the host.

Although these copepods are not, ordinarily, very dangerous to fish in their natural haunts, yet from the standpoint of fish-culture they are of considerable eco-

nomic importance. When once they make their appearance in our hatcheries they cause a great deal of damage and loss amongst the fish. Here conditions are ideal for parasitism. The ponds are small and large numbers of fish are crowded into them. Because of this situation the parasitic larvæ have very little trouble in finding their hosts. At the same time the current of water which circulates through the hatchery ponds is not swift enough to interfere with the movements of the parasitic organisms. It is therefore a matter of a short time before most of the fish become heavily infested with copepods.

While the young fish as well as the adults are attacked in the hatchery ponds, nevertheless it is mainly the adult fish which are most heavily parasitized. These are attacked by so many of the copepods that they are ultimately killed. It is by no means uncommon to find as many as two hundred and fifty copepods on one trout. Recently I found around five hundred copepods on the gills of a single chinook salmon. In such cases of parasitism the injury to the host is considerable. In the first place, the parasites suck enormous quantities of blood, thereby depriving the host of a large amount of nourishment. Secondly, when the copepods attach themselves, they injure the tissues of the host, thereby making it possible for injurious spores and bacteria to enter and set up secondary infections of a serious nature. And lastly, the injured tissues swell and develop into so-called "scar tissues," which interfere with the normal functions of the host. Taking all these facts into consideration, there is little wonder that fish succumb under the attacks of these parasites, particularly in hatchery ponds where conditions are just right for parasitism. In one Wisconsin hatchery the author found that in a single year about twelve thousand adult trout out of fourteen thousand kept in outdoor ponds died from the attacks of these copepods.

Many states have had this trouble for years, with very serious losses. The writer has devoted considerable

attention to the control of these parasites, and has recommended the following remedies in the state of Wisconsin. These have also been found useful in other states where the same type of parasitism has made its appearance.

1. When the water supply is polluted, sand filters should be installed at the mouth of the water stream as it makes its way into the hatchery ponds. The sand catches most of the free copepods before they enter the hatchery, thereby preventing them from attacking the fish.

2. The young fry should be given salt baths quite often. The salt solution kills the copepods during the early stages of attachment. At the same time this solution makes the fish more resistant to the attacks of the parasites.

3. Since the adult fish are the ones most heavily parasitized, it is better to do away with these as soon as possible and to keep only the younger fish for spawning purposes.

4. Inasmuch as the free-swimming stages of the copepods are strongly attracted by intense light, powerful arc lights should be erected at various points over the fish ponds. By means of fine gauze bags towed over the illuminated regions, a large number of the copepods can be gathered and removed.

5. The introduction of certain types of minnows into the hatchery ponds tends to keep the parasites down. These minnows feed on the free-living larvæ of the copepods, thereby destroying many of them before they have the opportunity of coming in contact with the proper host.

Another means of overcoming this sort of parasitism which has often suggested itself to the writer is, through breeding, to develop a strain amongst the hosts which would be practically immune to the attacks of the parasites. This appears to be possible when one considers the fact that under similar conditions the hosts show varying degrees of resistance to the parasitic organisms. Some

are attacked very lightly, while others become heavily parasitized. Doesn't it seem logical to speculate that through intelligent selection and breeding, one could develop resistant strains of fish, which would be attacked by so few of the parasitic copepods that the parasites would be almost a negligible quantity?

These remedies, of course, are not absolute, but they may help a great deal in reducing the loss of the fish. In cases of such parasitism there is no absolute cure known. A most desirable remedy would be one which would destroy the adult copepods while they are attached to the structures of the fish, without in any way harming the latter; but all attempts in this direction have thus far been without success. The hosts are so delicately constituted that they can withstand only a very slight change in their environmental medium. The adult copepods, on the other hand, can resist powerful chemical solutions by virtue of their resistant body walls. It is obvious that the weak link in the chain of the life-history of these parasites is the free-living period, and in view of this, the real solution seems to be quite clear. One must catch the organisms as they break out of the egg-sacs of the mother and kill them before they come in contact with their hosts. As with a good many of our modern diseases, "prevention before parasitism occurs" should be our motto, rather than "cure after parasitism."

SHORTER ARTICLES AND DISCUSSION

COLLINS'S REMARKS ON THE VIGOR OF FIRST GENERATION HYBRIDS

In a review of the theories regarding hybrid vigor Collins[1] has attempted to show that the two objections which were long upheld as precluding the possibility of dominance accounting for heterosis were without foundation. The suppression of deleterious factors, he considers, is adequate to account for the observed facts without considering the phenomenon of linkage.

The two objections which were raised against the hypothesis of dominance as a factor in hybrid vigor before the importance of linkage became generally known are as follows: (1) Dominance of independent factors would give an asymmetrical distribution to the progeny populations of those individuals which show an increase in growth when crossed. (2) Free assortment would make possible a recombination of all the dominant favorable growth factors into a homozygous fixed race which would not be reduced by inbreeding. Neither of these objections holds when linkage is taken into consideration. Collins believes that they also do not apply when linkage is left out of consideration.

Collins shows numerically and graphically that with a large number of factors involved the skew curve of the theoretical distribution of independent dominant factors approaches the type of the normal curve. He points out that, with characters dependent upon a large number of factors, only populations with larger numbers than have been dealt with statistically would exhibit any noticeable tendency toward skewness. This is a good point well brought out which previously had been neglected. But this would apply only to progenies which have a restricted range in comparison with their parental populations. In those cases where the range of the segregating generations with small numbers nearly equals the combined range of the original races as exhibited by the characters which show heterosis the number of main factors which govern the expression of this particular character can not be large. Therefore, if it were merely a mat-

[1] Dominance and the vigor of first generation hybrids. Amer. Nat., 55: 116–133, 1921.

ter of dominance without linkage, such distributions would be expected to show right-hand skewness. But they do not consistently do so.

In regard to the second objection, that of recombination of all favorable factors, Collins has given a large number of figures to show what was already well known, that with a large number of factors the chances for recombination are remote with the small progenies grown in experimental plots. It was not intended to maintain that pedigree cultures were adequate to show that such a recombination could not be made. The point in mind, if not clearly expressed, was that natural selection in isolated populations of cultivated plants had not brought about any noticeable approach to stability. In the hills of New England maize has been grown for long periods of time in isolated fields. Some varieties have probably been grown for at least fifty years without admixture. Yet these varieties when self-fertilized show as rapid a reduction in growth as other varieties which are lately the product of extensive hybridization.

There is an enormous difference in the possibilities for immediate recombination with and without linkage. To illustrate: with twenty independent factors the chance for the bringing together of all dominants in a homozygous state in one generation is theoretically one in 4^{20}. With the same twenty factors distributed by twos in ten different chromosomes, each being separated by ten units of crossing-over, the chance for recombination is theoretically one in 20^{20}. This is a difference in total numbers so vast as to be almost inconceivable. Working over long periods of time, linkage may not be a hindrance to recombination, as factors once brought together tend to stay together as firmly as they once resisted separation. Many cross-fertilized species in the wild whose age is measured in geological periods rather than years are stable. But cultivated forms even when isolated for a considerable time show no noticeable approach toward this condition. It seems reasonable to suppose that the arrangement of factors in the chromosomes has something to do with this state of affairs. Therefore, until the chromosome theory of heredity was developed, there was considerable plausibility to the older view that something besides mere dominance was responsible for heterosis.

Even so, I am perfectly willing to admit that there is no clear way of deciding the argument as to whether or not the old objec-

tions were valid. But how important is it now to make this decision? Linkage is a fact and must be taken into consideration. True, the evidence in support of the chromosome hypothesis from maize is not extensive. But hybrid vigor is a widespread phenomenon shown by many organisms. The dominance hypothesis applies to *Drosophila* as well as to maizė.

This failure to look outside of the corn field has led Collins to make certain statements to which I must take strong objection. He is inclined to believe that the suppression of deleterious factors is all that is involved in the vigor derived from crossing. This may be true for *Drosophila*, but there are many cases of wild species of both animals and plants as well as of naturally self-fertilized varieties of cultivated plants which show an unmistakable increase in growth after crossing. Take Naudin's *Datura* crosses which doubled in height, Kölreuter's *Nicotiana* hybrids which astonished their producer, the hybrid walnuts, both natural and artificial, and Gerschler's fish hybrids, to name a few notable illustrations. Collins himself has given us several good illustrations of remarkable vigor shown by hybrids of many varieties of maize from different parts of the world. Here it is clearly not a matter of suppressing deleterious characters. The parental types are normal, vigorous and perfectly capable of maintaining themselves in their own way. But crossing brings about a new combination of hereditary qualities. By utilizing the best from both parents the hybrid is able to obtain a surpassing development. As long as variation exists different individuals will have unlike germinal potentialities. Crossing tends to bring these different possibilities together. Dominance enables the offspring to take advantage of the more favorable factors. This is as true of domesticated races as it is for wild species.

Furthermore, there is abundant evidence that many factors are without effect unless working in consort. In plants, colors of various parts, and in animals, coat patterns, are conspicuous examples of this complementary action. These characters are possibly of no importance in growth, yet they illustrate a state of affairs which is probably of real significance. Crossing makes it possible to assemble the component parts.

As Collins says, to consider hybrid vigor as the suppression of deleterious heredity as compared to the bringing together of a greater number of favorable growth factors is, to a certain ex-

tent, merely a different way of looking at the same thing. But it puts the emphasis on the wrong side and is wholly inadequate to account for all the manifestations of hybrid vigor. It would be unnecessary to discuss this were it not for the fact that his way of looking at the matter leads him to think that there is no essential distinction between the Darwinian view of inbreeding as a process leading toward extinction and the more recent conception that the results of this system of mating depend upon the inheritance received. Collins says:

> Many of the older writers on heredity have held that inbreeding is a cause of degeneration. In avoiding ambiguous words "cause" is one of the first that must go. If forced to define their position this school would probably be content with the statement that degeneration is a necessary consequence of inbreeding, the intermediate step or nature of the process being unknown. Is this conception really at variance with the idea that degeneration results from the increased number of unfavorable recessive characters brought into expression by increased homozygosity? Does not this conception rather amplify the older, general and indefinite position by explaining how degeneration may be brought about? (P. 124.)

Leaving aside all question of definition of terms, let us consider the results of the two views when applied in practise. To say that abnormal and undesirable individuals appear after close mating is very different from supposing that such forms have their origin in the system of mating. Whether or not this is stating the matter fairly, breeding practises have been in accord with the latter view. As a result of inbreeding we now know that aberrant individuals bordering on the teratological often come to light. Along with these types which are truly degenerate in any sense of the term (but inbreeding has nothing to do with their origin) there are perfectly normal individuals which suffer in comparison with their more heterozygous parents in that they are only slower in growth, are not so resistant to unfavorable conditions and are not so productive. Inbreeding is solely a process of sorting out. Some bad material is brought to view which can be discarded. But along with this there is all the good material that was in the stock, and this can be used to rebuild a better breed than existed at the start. Before the era of Mendelism there was little conception that it was the stock that was at fault and not the system of mating. Even though it was the appearance of abnormal and bizarre forms which gave

the bad name to inbreeding in the past, the less vigorous offspring frequently resulting from inbreeding, although healthy. were also considered to be valueless for further propagation and were quickly disposed of.

This is still the belief and practise of live-stock breeders. Those who do not know the principles involved think that inbreeding has permanently injured the families with such weakened individuals. Equipped with the results of two decades of genetic investigation, we can say, "No! this is not so. *Nothing has been lost.* These less vigorous inbred individuals of no apparent worth have potentially great value." A widespread reception of this idea has possibilities of great practical outcome. Not to see clearly the important distinction which there is here between the present and former views is not to appreciate the real progress which the combined genetic research of twenty years has made along this line.

D. F. JONES

CONNECTICUT AGRICULTURAL EXPERIMENT STATION,
NEW HAVEN, CONN.

AN APPARENT CASE OF SOMATIC SEGREGATION INVOLVING TWO LINKED FACTORS[1]

SOMATIC segregation as an ordinary occurrence, and especially as a source of definite progeny ratios in subsequent sexual reproduction, seems highly improbable. The evidence connecting normal segregation and recombination with meiosis and fertilization is too strong. As a matter of occasional mitotic abnormality in heterozygous material, however, the question of somatic segregation is still open.

Any "bud sport" involving apparently simultaneous change of two or more non-allelomorphic factors is therefore of special interest, since the probability of its occurrence through two nearly simultaneous factor or point mutations seems very remote. Either deficiency mutation, which seems to mean (Bridges, 1917) the loss of a normally present portion of a chromosome, or the development or resolution of a condition of "duplication" (such as vermilion-sable duplication in *Drosophila melanogaster*; Bridges, 1919, p. 646) might produce the effect in question. So, also, might a process properly described as "somatic segregation," in which at some mitosis one daughter

[1] Paper No. 60, University of California, Graduate School of Tropical Agriculture and Citrus Experiment Station, Riverside, California.

cell received both halves of one mother-cell chromosome, while the other daughter cell received both halves of the homologous chromosome. Such a variation, then (Muller, 1920, p. 459), permits an almost certain decision between factor mutation and "mitotic irregularity."[2]

A "bud variation" apparently involving two linked factors was observed in a culture of *Matthiola annua*, at Riverside, California, in 1916–17. Unfortunately the factorial relations are not entirely clear, but the case seems decidedly significant nevertheless.

The plant in question occurred among progeny of a "slender" parent (25b–6–8–6; Frost, 1919). The slender type (S') is one of several aberrant forms evidently dependent on factors linked with the factor "for" single (normal) flowers (D). Slender parents have given (Frost, 1919) on the average about 32.5 ± 2.0 per cent. of slender progeny, most of the rest being "Snowflake" (normal). The constitution of the slender single parent mentioned appears to have been $S'D/s'd$. Both S' and D (or a factor completely linked with D) appear to be imperfectly recessive for a lethal effect; no functional pollen carries D,[3] and $S'S'$ zygotes appear to be non-viable, while $S's'$ zygotes are somewhat weak and probably are selectively eliminated before germination.

Plant 25b–6–8–6 gave the following progeny: slender, 18 or 19 (2 double, 1 undetermined, rest single); Snowflake, 25 (1 single, 24 double); total, 44. One plant was noted, at the age of about seven months, as having the upper main stem leaves like Snowflake, but the rest slender. When mature this plant had produced from one side of the main stem at least three primary branches, all slender and single, two at least yielding seed. The main cluster was stout, and, although its flowers seem not to have been noted as peculiar while in bloom, it produced persistent sterile pistils; at least two of these pistils were abnormally broad, each enclosing a cluster of petal-like parts. One

[2] This paper was written, aside from some revision of this second paragraph, before I saw Muller's paper here cited.

[3] All the singles of such a "double-throwing" race are therefore heterozygous for doubleness, while the doubles (dd) are sterile (Frost, 1915). A back cross of two Snowflake plants by pollen of 25b–6–8–6 (crosses 23ea and 23ea; Frost, 1919, table 36) gave about 22.4 ± 2.6 per cent. of slender progeny, including only 2 (or 1) doubles out of 26 slenders, while the Snowflakes were about half doubles. Evidently both eggs and pollen carried some factor or factors contributing to this puzzling result.

stout flowering branch, well above the others, evidently was similar to the primary inflorescence. Near the level of the uppermost of the slender flowering branches, on the opposite half of the stem, arose two stout branches, which bore Snowflake-like leaves and sterile double flowers.

It would seem that some change eliminating the factors *S'* and *D* occurred, probably in a single cell, at the growing point of the young main stem. The Snowflake double (*s'd*, *s'd*) cells resulting, perhaps because of their normally greater vigor of growth, gradually obtained the ascendency in a large portion of the stem. The primary inflorescence and the high branch beside it perhaps remained in a chimerical condition, while the two lower stout branches received the new type nearly or quite unmixed.

That the double flowers were somewhat abnormal[4] hardly lessens the force of the evidence in relation to the improbability of factor mutation. Even the two lower stout branches may have been periclinal chimeras, or the new factorial constitution may have been (as for example through a "duplication" shifting of chromosome material) somewhat different from that of a normal double. Plainly some change occurred that involved, nearly or quite simultaneously, two factors in linked loci some distance apart. This change was probably not factor (point) mutation. It may have been deficiency mutation, itself probably a mitotic abnormality, or it may have been some other abnormal shifting of a chromosome or a portion of a chromosome.

A further consideration is pertinent here. The slender form and at least one or two others, in arising (Frost, 1916, 1919) in very small proportions from the normal (Snowflake) type, show evident linkage phenomena which indicate segregation rather than immediate mutation. As has been suggested (Frost, 1919), the apparently mutant factor (as *S'* above) may be present in ordinary Snowflake singles, but concealed because of the

[4] The usual double flowers are "petalomanous" (de Vries, 1912, p. 330); that is, inside the sepals they consist of nothing but an indefinitely proliferated floral axis bearing numerous petals. The flower lives long after anthesis, and often develops into a short branch bearing secondary flowers in the axils of its leaves (petals). No trace of stamens and carpels can be found. These abnormal double flowers, on the other hand, had the four petals of the typical cruciferous flower, followed by an indefinite number of smaller curved petals probably representing petaloid stamens; finally, in some cases the central mass of petals seemed to arise from within a modified pistil, somewhat as in the case of the less abnormal flowers of the terminal cluster mentioned above.

action of an epistatic or inhibiting factor *I*. Thus the constitution of the Snowflake singles giving rare slender progeny may be *IS'D/is'd* or *DS'I/ds'i*. A serious theoretical difficulty seemed to arise in the apparent necessity for several specific "inhibitors" all giving the same "normal" type, and also for relatively frequent dominant mutations. Perhaps, however, the apparent mutation may usually consist in the development or disappearance of some condition of duplication in one chromosome of the pair concerned. Origin of apparent mutants through duplication of whole chromosomes, as seems to have been demonstrated for a remarkably similar series of mutant forms in *Datura* (Blakeslee, Belling and Farnham, 1920), seems to be precluded in these cases by the evident linkage phenomena.

HOWARD B. FROST

UNIVERSITY OF CALIFORNIA

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THE NEUROMOTOR APPARATUS OF PARAMECIUM

THE discovery of a neuromotor apparatus in *Diplodinium ecaudatum* (Sharp 1) and *Euplotes patella* (Yocom 2) confirmed by Taylor (3) leads me to expect similar conductile fiber systems in other ciliates. This expectation has been met in the

well-known ciliate *Paramecium*. The neuromotor apparatus of this organism consists of fine branching fibers in the periphery and the cytopharynx, converging to the neuromotor center.

In the periphery these fibers are connected to the basal granules of the cilia and also to the trichocysts. From these organelles they may be traced to the neuromotor center (Fig. 1, *n. c.*) located in the endoplasm just anterior to the cytostome. They

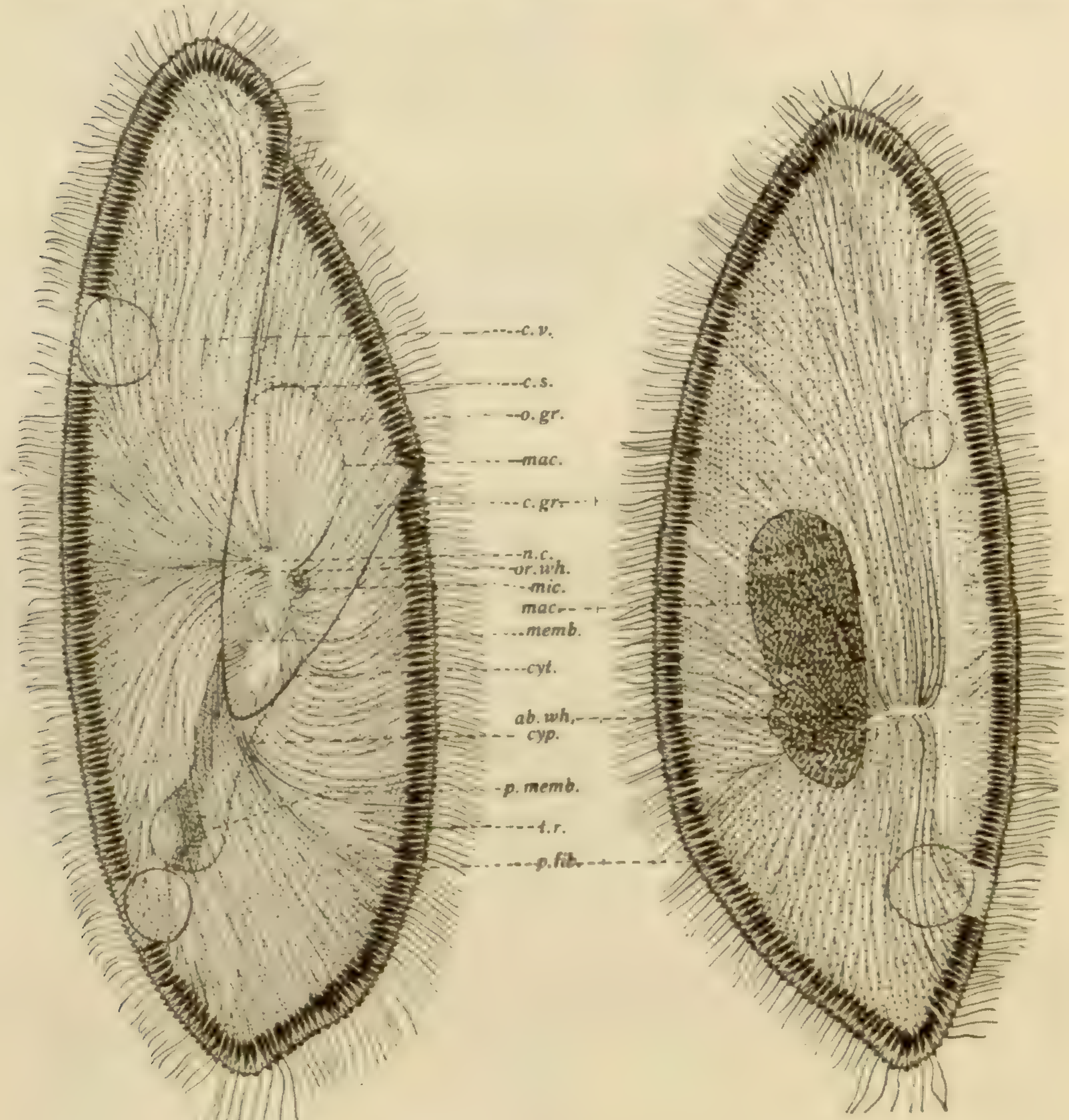

FIG. 1.

FIG. 2.

FIG. 1. *Paramecium caudatum*, diagrammatic sketch showing oral whorl of peripheral neuromotor fibers, the neuromotor center, ciliary grooves, trichocyst ridges, ciliary suture, cytostome and cytopharynx with anterior and posterior membranelle zones.

FIG. 2. *Paramecium caudatum*, diagrammatic aboral view, showing aboral whorl of neuromotor fibers.

Abbreviations: *c. v.*, contractile vacuole; *c. s.*, ciliary suture; *o. gr.*, oral groove; *mac.*, macronucleus; *mic.*, micronucleus; *n. c.*, neuromotor center; *or. wh.*, oral whorl; *memb.*, anterior membranelle zone; *cyt.*, cytostome; *ab. wh.*, aboral whorl; *cyp.*, cytopharynx; *p. memb.*, posterior membranelle zone; *t. r.*, trichocyst ridges; *p. fib.*, peripheral neuromotor fibers.

are arranged in whorls, one on the oral side, the other on the aboral side (Fig. 1, *or. wh.;* Fig. 2, *ab. wh.*).

The oral whorl is the more extensive. In the oral groove (Fig. 1, *o. gr.*) the fibers run obliquely caudad to the cytostome where they turn and converge obliquely cephalad to the neuromotor center. From other parts of the oral surface they run in gracefully curved lines directly to the neuromotor center.

The fibers of the aboral whorl converge in a large apex on the right, opposite and slightly posterior to the cytostome. Here they dip into the endoplasm and run direct to the neuromotor center.

The entire periphery of the animal is supplied with the diverging fiber ends of these two whorls. On the left side those of the oral whorl meet those of the aboral whorl about midway between the two sides. On the right the inner ends of the fibers of the oral whorl mingle with those of the aboral whorl near the converging apex of the latter.

Two sets of fibers connect the organelles of the cytopharynx with the neuromotor center. One, a fan-shaped set, runs in the right wall of the cytopharynx to the anterior membranelle zone. The other set consists of two fibers which run from the neuromotor center to the peristomal cilia around the cytostome and meet in the posterior margin. From here they run in the oral side of the cytopharynx and branch profusely into the posterior membranelle zone and the endoplasm. This posterior zone, the cilia of which beat in an opposite direction to those of the anterior membranelle zone, has not been previously described. The cytopharyngeal fibers are heavier than the peripheral fibers and may be seen in living unstained animals under oil immersion.

The trichocysts are arranged with reference to the peripheral neuromotor fibers in whorls. They reach the surface of papillæ which constitute interrupted ridges (Fig. 1, *t. r.*). The cilia, however, spring from longitudinal grooves. The grooves from each side of the oral surface meet in a series of V's, the apices of which lie in a line, the ciliary suture (Fig. 1, *c. s.*) which extends obliquely through this surface from the anterior to the posterior end.

Fibers were found in 2.5 μ sections connected to the basal granules of the cilia and running into the endoplasm. Khainsky (4) also found these fibers and called them ciliary rootlets. They are here interpreted as the ends of the peripheral neuro-

motor fibers. Similar fibers have been found connected to the inner ends of the trichocysts.

From the foregoing it is seen that the neuromotor system of *Paramecium* consists of fibers running from the neuromotor center to the membranelle of the cytopharynx and also from the same center to the basal granules of the peripheral cilia and to the trichocysts. Its morphology suggests that it is conductile in function adapted to coordinate the movements of the peripheral cilia and the cytopharyngeal membranelles.

The peripheral fibers were discovered in whole mounts fixed, stained and dehydrated in centrifuge tubes. The best stain was Heidenhain's iron alum hæmatoxylin. They were not seen with this stain when the animals were attached to the slide by egg albumen. However, contrary to Neresheimer (5), the distal fiber ends were sometimes seen in such preparations when Mallory's triple connective tissue stain was used. Complete cytological details were worked out only from the hæmatoxylin preparations.

This idea of staining non-distorted animals in centrifuge tubes resulted from micro-injection studies. It was found that animals survived such operations only when isolated in rounded drops. Blisters invariably formed when they were held flattened to the cover by water-glass surface tension (Taylor 3). An apparatus embodying the principle of Taylor's micro-injection pipette (Taylor 6) was constructed by means of which isolation was accomplished in such small drops that only a very limited movement of the animal was possible. To secure rounded drops the cover was coated with a thin film of oil as described by Barber (7).

Three kinds of experimental methods in attempting to demonstrate that the fibers are conductile were carried out as follows:

Grübler's methylene blue which stains nerve fibers in metazoan tissue (Wilson 8) gave negative results when injected into *Paramecium*.

An antero-posterior gradient was demonstrated as follows: The organisms were isolated in 4 per cent. to 6 per cent. alcohol, $\frac{1}{10}$ per cent. nicotine, 1 per cent. antipyrin, or 1 per cent. morphine hydrochlorate. In all cases the anterior cilia ceased beating at least ten seconds earlier than the posterior cilia and those of the cytopharynx. The animals did not disintegrate as do planarians and annelid worms in these solutions so that

the physiologically anterior end (Child 9) could not be determined. But the antero-posterior gradient is what one would expect in animals possessing fibers which conduct efferent impulses (Tashiro 10).

Contrary to Neresheimer (5) the animals are narcotized in these solutions.

Micro-dissection experiments showed that the coordination of movement of the cytopharyngeal membranelles is interrupted when the neuromotor fibers are cut. Those posterior to the cut beat slower and with smaller amplitude than those anterior to it. Extensive destruction of structures in the region of the neuromotor center or motorium destroyed coordinated movement of the peripheral cilia. In one case in animals isolated in gelatine four zones of cilia were seen. Those of one side beat in opposite directions to those of the other.

CONCLUSIONS

A complex fibrillar apparatus has been differentiated in *Paramecium*. It connects the membranelles of the cytopharynx and the peripheral cilia and also the trichocysts with the neuromotor center. Therefore, the morphology of this system suggests that it is conductile. Experimental data strengthens this morphological evidence; first, because the antero-posterior gradient that exists here is that which would be expected in an animal possessing a complex system of fibers which conduct efferent impulses from the anterior end to the neuromotor center; second, the micro-dissections indicate that coordinated movement of the cytopharyngeal membranelles is interrupted when neuromotor fibers are severed and coordinated movement of the peripheral cilia is interrupted when the neuromotor center is destroyed.

CHARLES W. REES

ZOOLOGICAL LABORATORY, UNIVERSITY OF CALIFORNIA

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A NEW MUTATION IN THE HOUSE MOUSE[1]

A NEW and distinct mutation in mammals is not a frequent occurrence, and therefore the record of a recent dilute form of the house mouse, allelomorphic to color and albinism, is perhaps justifiable.

The infrequency of mutations in mammals may be due to greater stability of the germ plasm than in such forms as insects, for example, *Drosophila;* or may be due to our lack of opportunity for examining as large a population of mammals as of insects; or possibly may be due to a more frequent lethal effect associated with mutations in mammals. Whatever the cause is, a tendency toward similar mutations in closely related groups of mammals is apparent and suggestive. The fact that a given type of mutation has occurred in one group is some promise that a corresponding mutation is possible and may occur in a closely related group. The pink-eyed mutation (giving PINK-eyed colored varieties) in mice has been known for some time. A similar mutation in rats was described recently (Castle, '14).[2]

[1] Paper No. 17, Genetics Laboratory, Illinois Agricultural Experiment Station.

[2] Castle, W. E., 1914, AM. NAT., Vol. 48, p. 65.

In both rats and mice, this gene greatly reduces the production of black and brown hair pigment, but leaves yellow undisturbed. The linkage of pink-eye to albinism in both forms gives added evidence that these genes are similar if not actually identical. A gene for pink-eye is also known in the guinea pig (Castle '14)[3], but it has not yet been established that pink-eye and albinism are linked in this form. It may be unsafe to homologize strictly the gene for pink-eye in mice and rats with the gene in guinea pigs which produces similar somatic effects. A deep red-eyed (almost black-eyed) yellow rat has also been described (Castle, '14).[4] The gene for red-eye is linked to the gene for pink-eye. This mutation has not yet been observed in mice. Brown varieties of mice and guinea pigs have been known for a long time. Brown rabbits (the Havana variety) and the brown roof rat (Patterson, '20)[5] are recent productions. While no brown variety of the common rat (*Mus norvegicus*) is known, the form should be possible and its discovery is only a matter of time and opportunity. A set of quadruple allelomorphs in the guinea pig includes intense color, dilution, ruby-eyed dilution and Himalayan albinism (Wright, '15).[6] Soon after these forms were found, Whiting and King ('18)[7] reported ruby-eyed dilution in rats, an allelomorph of both color and albinism. While Whiting and King used the same symbol, c_r, for this gene that Wright used for ruby-eyed dilution in guinea pigs, they point out that the somatic effect is somewhat different. The two cases of ruby-eyed dilution may not be identical, for it is conceivable that numerous dilution effects in the color-albino series are possible. Ordinary color dilution still remains to be found in rats, in order to make up a series of color allelomorphs as elaborate as in the guinea-pig. Similar examples of apparently parallel or identical mutations in closely related groups can be shown in other animals, as in the color varieties of the horse and the ass, for example, and in the case of the *Drosophila* species.

In an effort to homologize the genes which affect the quality and distribution of hair pigment in the mouse, rat, guinea pig and rabbit, I was impressed by the dearth of color allelomorphs in mice, such as cause the various grades of dilution in the rat

[3] *Loc. cit.*
[4] *Loc. cit.*
[5] Patterson, J. T., 1920, *Science*, N. S., Vol. 52, p. 249.
[6] Wright, S., 1915, AM. NAT., Vol. 49, p. 140.
[7] Whiting, P. W., and King, H. D., 1918, *Jour. Exp. Zool.*, Vol. 26, p. 55.

and guinea pig. It appeared that similar changes in the mouse were possible and could be found, if persistently sought. With this general thought in mind, I attempted by corresponding with fanciers *et cetera* to locate dilute mutations in mice, thinking that the dark red-eyed yellow mutation (parallel to the type found in rats) or the dilute forms allelomorphic to color and albinism (parallel to the guinea-pig and rat series) might be possibilities. My correspondence with fanciers brought no results, but a dilute mutation appeared from a rather unexpected source. On August 31, 1920, Mr. J. E. Knight of Weldon, Illinois, who exterminates rodents from corn cribs, poultry houses and the like, and who has much opportunity to examine a large number of these mammals, brought to my laboratory a young male mutant mouse which he had captured in a corn crib, located on a farm seven miles from the nearest town. This animal on a first and cursory examination, gave the appearance of being an ordinary black-eyed white in which the hair was apparently very slightly stained or dirty. Realizing that such a form would mean a double (and therefore much more improbable) mutation from the wild, in which both dominant and recessive spotting occurred simultaneously, I made a more careful examination about one month later and found that the hair had become darker. I have since learned that it is characteristic of this form to be practically white on the first pelage, but the dorsal hair eventually acquires a brownish shade,—a little lighter than an ordinary pink-eyed brown with a slight dull yellowish cast. There is no clear evidence of an agouti pattern, the base of the hair being light and the apical portion pigmented. The ventral surface is almost white, at least in the presence of agouti. The eyes at birth are somewhat less heavily pigmented than the wild. This difference persists for some time, but when the mutant is full grown I am not sure I can distinguish the eye from the wild type. Dark pigment is quite pronounced in the skin of the ears and scrotum, in which respect this mouse differs from the ruby-eyed rat. The dark eyes and yellowish tinge in the hair at first suggested that the mutation was similar to the dark red-eyed yellow rat. Recent matings have, however, demonstrated quite clearly that it is a third allelomorph in the color-albino series, and may therefore be homologous to the ruby-eyed dilute rat which is allelomorphic to color and albinism.

While the hair of the mutant mouse is lighter and the eyes are

apparently darker than the ruby-eyed dilute rat, the genetic behavior agrees quite closely in both forms. Nevertheless it is hardly safe to insist that these two mutations are identical, for there may be numerous possible grades or conditions of the color gene. We are also unable to prove that they are different, for the genes may be identical, but simply give different somatic effects since the residual inheritance in the two forms can not be the same. If a new dilute type of mouse can be found which is more like the rat in both genetic behavior and somatic appearance, then we shall be able to state with more assurance that the present mutation is not identical with the dilute rat. Until that event occurs, we can only regard these dilute color mutations in the rat and mouse as samples of a series of possible mutations in the color gene (cf. the red, white, eosin, cherry, et cetera series of multiple allelomorphs in *Drosophila melanogaster*). A similar interpretation applies in any attempt to homologize the members of the guinea-pig series with those of the rat or the mouse series.

Three types of crosses between the mutant male mouse and other color varieties have been made as follows:

1. Mated to homozygous blacks, all of the F_1 offspring were wild gray. In the F_2 the mutant and other expected forms segregated out.

2. Mated to pink-eyed spotted brown, all of the F_1 offspring were wild gray. In the F_2 the expected forms occurred including the mutant type. We have not yet had opportunity to identify the mutant form when homozygous for pink-eye.

3. Mated to albinos, all the F_1 offspring were of the *mutant* type, that is, they are white at birth with eyes rather less heavily pigmented than the wild type. As they grow older, the hair soon approaches the mutant color type, but I can not yet state whether the mutant hair color is incompletely dominant, as in the case of the ruby-eyed dilute rat and guinea pig.

From present indications, this new dilute mutation is certainly recessive to color, and I am inclined to believe it will prove to be incompletely dominant to albinism. The three genes (color, color dilution, and albinism) probably form a series of triple allelomorphs. I shall designate these genes by the symbols C, c^d, and c, respectively. The mutant mouse is homozygous in agouti, black, dark-eye and self pattern, and therefore represents a single factor difference from the ordinary wild type, from

which it arose. The new gene should prove to be linked with dark-eye, like its allelomorph, albinism. Since it occupies, in a scale of dominance, an intermediate position between color and albinism, the mutant should give a coupling series when mated to albinos carrying pink-eye $\left(\mathrm{F}^{1} = \frac{c^{d} \qquad P}{c \qquad p}\right)$ but a repulsion series when the same mutant is mated to pink-eyed colored individuals $\left(\mathrm{F}_{1} = \frac{c^{d} \qquad P}{C \qquad p}\right)$.

J. A. Detlefsen.

GENITAL ORGANS OF HERMAPHRODITIC FUR SEALS

The resumption of commercial killing of the surplus and useless males of the fur-seal herd resorting to the Pribilof Islands of Alaska furnished an opportunity to study the life history and anatomy of this group of mammals. After a lapse of six years, killing was begun again in 1918 by the Bureau of Fisheries. Upwards of 33,000 males, mostly young, were secured and their skins preserved for sale by the government. Two hermaphroditic animals were killed among this large number and the writer had an opportunity of examining the sexual organs of both. Such abnormalities of the species have not previously been recorded and since they are rarely found among mammals of any species it seems desirable to note the occurrence with a brief description. The organs of both animals have been deposited in the United States National Museum.

Normally the female Alaska fur-seal has two kidney-shaped ovaries located just forward of the pelvis and loosely invested in the folds of connecting ligaments. Blood vessels, ureters, fallopian tubes and uteri are attached to the same folds. The ureters pass above the genitalia but bend down below to reach the tip of the bladder. The uterus is bicarinate, an ovary being attached to the distal end of each horn. It is pretty well determined that each side functions alternately every other year. The horns unite in the median line and the vagina continues to the exterior, a distance of about 20 cm. Attached to the lower side of the vagina is the pear-shaped and very muscular bladder. The urethra leading therefrom is deeply embedded in the muscular walls of the lower vagina as it passes to its point of discharge near the exterior.

The first of the hermaphroditic animals to be described was found at the Northeast Point hauling grounds of St. Paul Island on August 6, 1918. It was four years old and thought to be a male by the external characters of the head. It was not discovered to be bisexual until skinning had started and the mammary glands were found fairly well developed and containing a small amount of milk. This is one of the first characters to be noted when a female has been killed. As the native skinner thought this had happened, my attention was immediately called to the matter. Two or three dozen females are unavoidably secured when large killings are made in the manner followed in 1918 and it was thought that this was one of these unfortunate accidents. But when the penis opening on the abdomen was seen in the usual place is was known that a freak had been found and its organs and skull were preserved.

The right ovary was found to be smaller than normal and pear-shaped instead of flatly oval and it was entirely divested of the usual covering membrane. Its fallopian tube was thicker and fleshier than usual. But the left ovary was much larger than normal and the membrane was firmly attached all over its surface by adhesions. Both horns of the uterus were normal in shape but smaller than usual in a four year old female. The walls of the vagina leading backward from the uterus were extremely heavy and firm. The opening grew smaller and smaller posteriorly until it reached the point of junction of the vagina walls and the penis. Then it followed the latter organ forward on the ventral side as a small duct. The opening to the exterior was near the distal end of the penis.

There were no testes; rudiments even could not be found. But the penis was well developed and in the normal position. The os penis was only about one fourth as large as would be found in a male of equal age. The cartilaginous continuation of this bone and the continuation of the walls of the vagina were one and the same.

The urinary system was normally developed, the bladder being attached to the vagina. The urethra followed the penis forward on the side opposite from the duct of the vagina. The muscles for the retraction of the penis were well developed.

It would seem that the presence of the female reproducing organs would preponderate in affecting other characters of the animal such as the skull. (This is widely different in the two

sexes.) But such was not the case. Although possessing decided features of both sexes the skull resembles, far more, one of a male of the same age.

The other specimen found in 1918 was very much less interesting. It was secured on Lukanin field, St. Paul Island. The organs were brought to me after the killing was over in a somewhat mutilated condition but the relations seemed to be about as follows:

There was a pair of testes, apparently in the usual position of ovaries. The spermatic cords united above the neck of the bladder and seemed to discharge through a large blind glandular-walled pouch. This latter was taken to be a pathological vagina and was all that remained of the female system. The bladder was normal in size and the urethra passed straight backward from it as in the female. A rudimentary penis two centimeters long with a minute bone projected posteriorly beneath the anus and the opening of what was taken to be the vagina. The urethra discharged through this penis. This animal was probably a two year old, but its skull was not preserved and the external characters were not reported to me.

An old native sealer once told me that he had seen a half male and half female seal about five or six years old. It was as large as a male of that age but had the beautiful soft pelage of the female or the young male. In this connection it is worth while to recall an attempt which was made with poor success in 1896 to castrate pups. It might be that he saw one of these but it is more likely that he saw a hermaphrodite. It has been recommended that castration be attempted on animals older than pups, say two year olds. If successful it is probable that they would develop to the size of the full-grown male but would retain the very valuable fur of undeveloped males or of females. As the males are when full grown four to six times as large as the females, the pelts should be proportionately increased in value.

G. DALLAS HANNA

CALIFORNIA ACADEMY OF SCIENCES,
SAN FRANCISCO, CALIF.

INHERITANCE OF BELTING SPOTTING IN CATTLE AND SWINE

IT has long been noted that when two belted individuals are bred together only part of their offspring show true belts.

Taking the Dutch Belted breed in cattle, although the new belted Galloway (Ashton) may later serve just as well, we find that in practically all pure-bred herds there appear individuals with imperfect belts and more often those that are pure black.

Kuiper has furnished us with the most promising results so far. He shows that the characteristic markings of the Lakenvelder or Dutch Belted breed may be obtained by crossing within the breed or by crossing with spotted cattle. In his experiments a belted bull was bred to 55 Holstein-Friesian cows and produced as offspring 27 belts, 24–25 self-black, and 3–4 spotted. The identity of one calf was doubtful.

To explain these results he takes two pairs of allelomorphic factors L-l for belt, epistatic over E-e for self, and a repulsion between L and E in the reduplication series 1–7–7–1. A fairly high correlation exists between white feet and wide belts. This correlation agrees closely with Walther's work on horses.

Kuiper's work may be criticized on the fact that he has no definite grounds for assuming the presence of allelomorphic factor pairs. He does not assume a factor for white spotting that will take in all parts of the animal.

In crosses between the single colored reddish-brown Netherland cattle (Richardson) a very few self-color individuals were produced. Crosses between the reddish-brown Netherland cattle (Kiesel) and Holstein-Friesians produced in the F_1, 90 well spotted, 84 medium spotted, and 6 self-colored individuals, showing that the dominance of either character was not complete. Crossing the F_1 individuals together gave 22 self-colored and 29 spotted. These results show that the Holstein-Friesian markings are of a heterozygous type and Holstein-Friesians when bred to Dutch Belted gave practically a 1–1 ratio. Assuming that all possible factor combinations were made this would easily prove that the belting in the Dutch Belted breed is a simple heterozygous condition and explains the appearance of offspring other than belts when belts are bred together.

In Hampshire swine there seems to have been considerable selection within the breed. Originally they were white or black and white (Youatt). Later, selection brought them to their

present color either black or black with a white belt (Day). White spotting other than belts appear, also an excessive white belting condition covers all but the extremities of the ears and the tail. Spillman states that ten per cent. of the progeny of registered individuals are without belted areas. He supposes two types of belting (*a*) homozygous, occurring very rarely, and (*b*) heterozygous, occurring as the common type. In crosses of other breeds on Hampshires (Simpson) (Severson) the spotting condition proved to be heterozygous, for when bred to recessive colored breeds the ratio of belts to non-belts was 11–10 (expected ratio 10.5–10.5).

Summing up all evidence so far on the inheritance of belting spotting one would be safe in saying that this character is due to a single heterozygous factor pair Ss for white spotting.

G. B. DURHAM

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STANDARDIZED MICROPHOTOGRAPHY

THIRD CONTRIBUTION: THE EXPOSURE FACTOR

THE great convenience to be derived from the use of only two tables of factors is so apparent that anybody who is at all familiar with microphotographic work should not for a moment

hesitate to spend some time in the preparation of such tables. It is, of course, natural that differences in the source of light, optical equipment and ray-filters necessitate the preparation of new tables. Our own outfit being one of the standard Bausch and Lomb apparatus with a Zeiss microscope and Cramer ray-filters, it occurred to me that those possessing a similar equipment might be spared the tediousness of preparing an exposure table if our table of exposure factors were published with such instructions as would permit of identical arrangement of the apparatus. Moreover, it will be remembered that these factors are dependent upon the source of light, substage position, magnification, and numerical aperture of the objective, and that other conditions being identical, exposure varies as the square of the numerical aperture.

A word must be said about the use of the substage condenser. The position of the condenser indicated in the table is such as to give the greatest detail without *apparent* bad effect on definition. As is well established, the depth of focus in a microscopic objective depends upon its numerical aperture. The greater the latter, the smaller the depth of focus. Since definition increases with numerical aperture, one has to sacrifice the one or the other. From a practical point of view depth of focus is often more desirable than perfect definition. The numerical aperture of an objective may be conveniently cut down by increasing the distance between the substage condenser and the objective. This can be done with safety only to a certain point, which I called the optimum, and beyond which definition is visibly impaired. I hope to be able later to return to this subject in greater detail. Meanwhile, we may state as a general rule that the higher the magnifying power of an objective, the sooner the optimum will be reached. With other words, the substage may be lowered a great deal more in low-power than in high-power objectives. In the following table the position of the substage is indicated in millimeters, assuming that it is at zero when moved up as far as it will go. In the Zeiss microphotographic stand the substage condenser has a numerical aperture of 1.40, and when placed at zero the surface of its upper lens is still 0.8 mm. below the surface of the microscopic table.

The source of light for which the table of exposure factors holds good is the Bausch and Lomb Microprojector No. 4301 with hand feed arc lamp and rheostat for 4.5 amperes, 110 volts,

D.C., placed so that the distance between the uprights carrying the projector and the microscope table is 20 inches, while halfway between the projector and the microscope a water cell is placed for the absorption of heat rays. The projector has a movable condenser of its own. The position of this condenser influences the intensity of light. Our table is made for such a position of this condenser that the light on the focusing screen is brightest. It is not the same for every objective and must be found by experimenting, with the aid of an assistant, who moves the condenser while the observer watches the field. For this experiment the microscope substage is best lowered about 2 mm. to insure even illumination. When the position of the condenser of the projector has been found and marked on the mounting,

TABLE OF EXPOSURE FACTORS FOR ORTHONON PLATES WITHOUT RAY-FILTER, USED WITH A B. & L. OPEN ARC D.C. 110 VOLT MICROPROJECTOR AND AN ABBE SUBSTAGE CONDENSER WITH N.A. 1.40.

Linear Magnification.	B. & L. Achromat E. F. 32 N. A. 0.10 Substage completely removed.	B. & L. Achromat E. F. 16 N. A. 0.25 Substage lowered 8 mm.	Zeiss Apochromat E.F.16 N. A. 0.30 Substage lowered 8 mm.	B. & L. Achromat E. F. 8 N. A. 0.50 Substage lowered 5 mm.	Zeiss Apochromat E. F. 8 N. A. 0.65 Substage lowered 5 mm.	B. & L. Achromat E. F. 4 N. A. 0.85 Substage lowered 4 mm.	Zeiss Apochromat E. F. 4 N. A. 0.95 Substage lowered 4 mm.	B. & L. Achromat F. 1.9 N. A. 1.30 oil immersion Substage lowered 1.5 mm.	Zeiss Apochromat E. F. 2 N. A. 1.30
25	0.0005								
30	0.0007								
40	0.0013								
50	0.0020								
75	0.0045	0.013	0.011						
100	0.0080	0.024	0.020						
125		0.037	0.031						
150		0.054	0.045						
175		0.073	0.061						
200		0.096	0.080						
250		0.150	0.125						
300		0.216	0.180	0.108	0.090	0.048	0.040		
350		0.294	0.245	0.147	0.122	0.065	0.054		
400		0.384	0.320	0.192	0.160	0.085	0.071		
500				0.300	0.250	0.133	0.111		0.040
600				0.432	0.360	0.192	0.160		0.058
700				0.588	0.490	0.262	0.218		0.078
800						0.351	0.284		0.102
900						0.432	0.360		0.130
1000						0.532	0.444		0.160
1250									0.250
1500									0.360
1750									0.490
2000									0.640
2500									1.000
3000									1.440

the experiment need never be repeated again. The table itself applies only to an Orthonon plate used without ray-filter. For use with Cramer's rayfilters the factors given in this table must be multiplied by the factors shown in my first paper.

The so-called "Pointolite" is an excellent source of light for low powers. It requires about 5–10 times longer exposures. In our laboratory we use the Complete Illuminating Apparatus with D.C. open arc. After some experimenting it was found that the most satisfactory arrangement of this illuminating system is when the arc is moved forward to within 2½ inches from the first condensing lens, a water cell placed between second and third condensing lenses, in contact with the mounting of both, and the distance between the third and fourth condensing lens fixed at 15 inches, while the distance from the latter to the microscope table is 7½ inches. Under these conditions the exposure is double that obtained with the microprojector for which the table in this paper is given. I omit giving a separate table for this system, because any one may obtain it in a moment by simply doubling the values given here. But it must be remembered that change in the arrangement of the illuminating system, no matter how small the deviation, will result in wrong values.

ALEXANDER PETRUNKEVITCH.

YALE UNIVERSITY

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EXPERIMENTAL STUDIES ON THE DURATION OF LIFE

I. INTRODUCTORY DISCUSSION OF THE DURATION OF LIFE IN DROSOPHILA [1]

RAYMOND PEARL AND SYLVIA LOUISE PARKER

SUCH quantitative knowledge as exists of fundamental principles in the general biology of the duration of life has, in the main, been derived from an examination by purely statistical methods of human mortality records. Of course a good deal of information about the biology of death and duration of life of a general and non-quantitative character has been gained from experimental work on lower organisms. This literature has recently been reviewed by one of us (Pearl (1) to (7) inclusive). But the outstanding fact is that most of the existing *quantitative* data about duration of life are purely statistical, and derived from man as material.

The statistical method of acquiring knowledge of natural phenomena has a number of distinct and important limitations (cf. Pearl (8)). It is the settled policy of this department to check every conclusion drawn from purely statistical methods by an independent experimental investigation of the same problems, wherever in the nature of the case this is possible. Most problems of human vital statistics can not, in the nature of the case, be investigated experimentally, in any direct way with man himself as material. Probably this is chiefly the reason why all of the immense mass of data collected, and work done upon vital statistics has contributed so little in the

[1] Papers from the Department of Biometry and Vital Statistics, School of Hygiene and Public Health, Johns Hopkins University, No. 45.

way of general principles to the science of biology. In outlining the plans of the department at the time of its inauguration provision was made, as a major element in the whole organization scheme, for experimental work on the duration of life, to parallel as closely as possible, in respect of its problems and foci of interest, the statistical work of the department. The present paper is the first of a series which will appear dealing with the experimental side of our work.

Originally it was planned to use mice as the material for experimentation on the duration of life, and a large and flourishing colony was bred up in accordance with the most critical genetic standards for experimental material. Just as the colony was ready to start definitive experimentation with, an accident completely destroyed it.

It was then decided, after advising with a number of persons, notably Professor T. H. Morgan and Dr. Jacques Loeb, to take up *Drosophila* as material for the extensive program of experimental work which we had planned. This organism has the great advantage over any other which could be used, that its genetic behavior and potentialities are more thoroughly understood than those of any other animal, thanks to the epoch-marking researches of Morgan (9) and his students. It has the further great advantage that under certain conditions, which we now rather clearly understand, its duration of life, both in respect of means and of the l_x or d_x distributions of a life table, is extraordinarily like that of man, with one day in the life of the fly corresponding roughly to one year in the life of man.

The first paper in the series aims to present, as a background of reference for further contributions, the following essential items:

1. A brief review of what has been noted by previous workers regarding duration of life in *Drosophila,* and other insects in so far as the observations are quantitative in character.

2. The details of our material and methods of exper-

imentation, which are critically standardized and have been used in the work which will be described in subsequent papers.

3. The general form and characteristics of the mortality curves of *Drosophila*, presenting mortality tables for certain strains.

4. The influence of certain phases of the experimental technique employed upon the results.

Specific problems regarding the duration of life will be presented and discussed in the subsequent papers in the series.

Literature

The earliest mention we have found of observations on the duration of life of *Drosophila* is a casual reference in a paper by Moenkhaus (10) published in 1911 in which he makes the following statement in connection with egg counts: "We have kept females alive 153 days." There are no details of any kind, as to conditions or numbers involved.

The first paper to make more than casual reference to the duration of life of *Drosophila* is a paper published in 1913 by Hyde (11). In studying fertility and sterility in different strains of flies he found two strains which differed to a marked degree in respect of length of life, and made crosses to study the behavior of the shortened length of life of the mutant "truncate" in heredity. His numbers are small, but they show the characteristic increased vigor of F_1 hybrids. The shorter average age of the lumped F_2's indicates that there have segregated out in the F_2 generation some short-lived flies. His data, however, do not give, or allow us to get, separate averages for the truncate F_2's and the normal winged F_2's.

His data are summarized in Table I.

In 1914 Baumberger (12) published a paper in which he gives data on the length of life of different orders of insects without food as affected by different constant temperatures, and by exposure to two different temperatures. Since the insects were caught in a net as imagoes the total

longevity is not known, so that the results have little significance from the standpoint of exact studies. The 359 insects had at 72° F. an average longevity of 4.8 days with a maximum of 15 days, at 62° F. an average of 6 days with a maximum of 23, and at 42° F. an average of 10.9 days with a maximum of 39. For the second part of the experiment 184 larvæ of the oak-tree moth were used. The results are too conflicting to allow one to draw any definite conclusions.

TABLE I

HYDE'S DATA ON INHERITANCE OF DURATION OF LIFE IN DROSOPHILA

Type of Flies		In-bred Wild	Trun-cate	F_1	Truncate ♂ × Inbred ♀ F_2	Recip-ro-cal F_2	Total
No. of Flies.		191	272	42	128	89	722
Mean duration of life in days.	♂ and ♀	37.4	21.4	47.0	29.5	29.3	.....
	♂	40.5	26.9	47.8	32.8	31.1	.....
	♀	34.5	18.5	46.4	25.9	27.3	.....

In 1915 appeared Lutz's (13) paper on natural selection in which he finds in each sex a slight negative correlation between the length of adult life and the duration of the embryonic periods. The distributions which he has for normal length of adult life with varying temperature give the 250 ♂s an average duration of life of 36.3 days, and the 263 ♀s an average of 28.9 days. He also gives distributions in hours of duration of life of flies which were given water but no food, and the correlations of duration of life of these starved flies with wing measurements.

During 1916 and 1917 Loeb and Northrop (14–16) published a series of papers on the effects of food and temperature on duration of life in *Drosophila*. The first preliminary paper in 1916 gives the duration of life of cultures of *Drosophila* in water and in cane sugar at temperatures from 28° to 9° C., showing a temperature coefficient for the duration of life of about the order of magnitude of that of chemical reactions, namely of about 2 for a dif-

ference of 10° C. The averages were much lower than those found by Hyde and Lutz because, of course, of the inadequate food. At 19° C. the culture in water had an average duration of life of 4.1 days and those in 1 per cent. cane sugar of 12.3 days. In 1917 the experiments were repeated, using sterile flies on 2 per cent. glucose agar which was found to be a more adequate food. Similar results were obtained, getting a similar coefficient for the duration of the larval and pupa stages, and finding that the ratios of the duration of the three different stages remained approximately constant for the different temperatures. The averages here of the life of the imago are more of the order of those found by previous workers, 228 flies at 30° lived an average of 13.6 days, 70 flies at 25°, 28.5, and 49 flies at 20°, 40.2. Later in 1917 they published another paper in which they give 92.4 days as the average duration of life of 143 flies at 15°, and 120.5 days of 105 flies at 10° C., together with the frequency distributions from which the averages were obtained. They also present results with different food mixtures, and for the two sexes separately, finding that isolated males live a little longer than isolated females, or than the males when mixed with females.

In another paper in the same issue Northrop (17) gives the results of some experiments undertaken to determine the effect on the duration of life of the imago of prolonging the life of the larva by inadequate feeding (omitting yeast for different lengths of time). In this way the embryonic periods were prolonged from 8 to 17 days, but the duration of life of the adult remained the same in every case, ranging between 10.5 and 11.9 days at 27.5° C., at which temperature the four experiments, involving 644 flies, were performed.

In a recent paper Arendsen Hein (18) gives a few observations on duration of life in the meal-worm *Tenebrio molitor*. Thirty-two male beetles lived an average of 60 days, with a range from 39 to 113, and 32 females averaged 111 days, with a range from 89 to 132 days.

MATERIAL AND METHODS

The flies furnishing the data set forth in this paper belonged to five different basic laboratory stocks or strains of *Drosophila melanogaster*. Four of these stocks were obtained from Professor T. H. Morgan in December, 1919, and have been bred continuously in this laboratory since that time. The original individuals of the fifth stock were collected by one of us (R. P.) as wild flies at Eagle Point, Lake Memphremagog, Vermont, in the summer of 1920.

The stocks may be listed as follows:

1. *Old Falmouth.* Wild type fly, long bred in Morgan's laboratory. More inbred than 2.
2. *New Falmouth.* Wild type fly bred for about 6 months in Morgan's laboratory before we got our sample of it.
3. *Sepia.* A mutant stock carrying one third chromosome mutation, sepia eyes, in homozygous form. Other characters wild types. (Morgan.)
4. *Quintuple.* A synthetic stock, carrying five second chromosome mutations, each in homozygous form, as follows: Purple, arc, speck, vestigial, and black. Other characters wild type. (Morgan.)
5. *Eagle Point.* Wild type collected in summer of 1920, and since bred in this laboratory.

An account of the second chromosome mutations mentioned will be found in Bridges and Morgan (19) and Sturtevant (20). The discovery of the mutation sepia is noted by Muller (21).

The stocks are carried along in the laboratory in pure mass cultures in half-pint milk bottles. Those in an experiment on duration of life are tested relative to this character in one ounce shell vials.

The flies are all kept on a standard food mixture made up fresh each day, according to the following method:

For each 100 c.c. of water add 2 grams of agar-agar. Boil the agar and water until the agar is thoroughly dissolved. For each 100 c.c. of solution add 100 grams of ripe peeled mashed bananas. Boil five minutes. Pour into bottles which have been well heated in oven (or sterilized in autoclav). In the breeding bottles pour a layer 3/4 inch deep; in duration of life bottles a layer 1/2 inch deep. When

the food has partly cooled sprinkle on top of food the smallest possible amount of pulverized dry magic yeast (shaken from a can with one pin hole in cover). Put in breeding bottle a folded square of filter paper, and stopper with cotton batting.

The purpose of the filter paper is to furnish a dry place for the larvæ to crawl up and pupate on, and also to absorb some of the excess moisture which often forms on top of the food from the growing yeast. Filter paper has not been used in the small duration of life bottles, since no young are pupating there, and since it furnishes too many hiding places for the flies in the frequent transfers which have to be made in the duration of life tests. Excess of moisture on top of the food may become a source of error in duration of life experiments, because flies may drown in a small drop of water. Throughout our work we have been constantly on guard against this source of error and have tried a number of plans, with varying degrees of success to eliminate it entirely. Some of these experiments will be reported on in detail later on in this paper. In general it may be said here that this source of error from flies drowning need never be a significant one if due precautions are taken. We know that it has not been in our work.

Occasionally the yeast becomes too active at the edge of the food and causes the whole food mass to rise in the bottle. In an attempt to eliminate this accident yeast in dilute solutions was added to the boiled bananas and agar and was sprayed on top of the food. The results were not particularly favorable. The pulverized dry yeast added in the most minute quantity possible is the most satisfactory standard method yet found. We expect to continue attempting to get the food conditions more and more nearly ideal and identical in every experiment, but we feel reasonably certain that in all of the experiments we shall report, even including the very first in point of time, the precautions taken to standardize food and to guard against accidental death were sufficient to insure statistical accuracy in the results. Whatever environ-

mental differences in respect of food have existed in our experiments have been randomly distributed among the different groups in any given experiment. We have had very little trouble at any time with moulds in the cultures, the frequent transfers in the duration of life bottles in an experiment preventing them from getting any start.

The stock bottles holding reserve stocks of flies have been kept at the varying temperature of the room, but all experimental flies have been kept in electric incubators at 25° C., in which recording thermometers have been placed to insure that no fluctuations of temperature have occurred without our knowledge. All the experiments on duration of life and their results recorded in this paper have been carried through at the constant temperature of 25° C. We have settled on this as a normal for this particular element of the environmental complex.

During the first year of the experimental work no attempt was made to keep the different generations separate in the stock bottles, the process being merely to keep enough bottles (generally 4) of each stock to insure always having pupæ and newly emerging flies on hand for any matings and experiments to be started. Each week all the flies old and young together from the oldest bottle of each stock were transferred to a fresh bottle. In this way each bottle was kept 4 weeks and there were always on hand bottles with flies in all stages of development.

In January, 1921, it was decided that it would be desirable to keep the generations separate in the stock bottles. All stock bottles were emptied on January 11, and flies in the stock bottles on January 14 were arbitrarily called generation O. From that time on the procedure has been to empty out all the parent flies from each stock bottle 7 days after the bottle was started (when there are usually a large number of larvæ and some pupæ formed). The bottles are then left for 7 days longer, during which time enough flies emerge to start a fresh bottle for the next generation. Several bottles are kept of each stock, as

before, to insure always having on hand newly emerging flies with which to start experiments.

When any experiment is to be started flies are taken from the stock bottles and etherized within 4 hours of emerging (usually sooner), before the wings have unfolded, so that they are surely virgin. Matings are made up as desired, putting the mated flies in half-pint milk bottles in the incubator. The parents are taken from the mating bottles in 8 or 9 days (before any young begin to emerge), and removed to a second mating bottle if a larger sample of progeny is desired than can be obtained from one bottle. As the offspring begin emerging they are shaken out every morning from the mating bottle to a small shell vial. Thus all the flies in a small bottle are the same age, and are properly labelled with mating number and date of emergence. Then every morning all these small bottles are looked over and those with dead flies separated out. After all have been looked over, the live flies in the bottles which have dead ones are shaken across to fresh bottles, the dead flies taken out and sexed, and all the pertinent facts as to duration of life, etc., recorded on printed blanks, from which the records are later (when all the flies of an experiment have died off) coded, punched on Hollerith cards, and sorted and tabulated by Tabulating Machine Company electric sorting and tabulating machines. Flies from any small bottles which are not changed (because of dead flies) within five days of the last previous transfer are transferred on the fifth day to fresh food. The physical manipulation is too great with the numbers we desire to use to admit of changing all the bottles every day, which would be the ideal way to keep food conditions absolutely identical for all the flies. Changing every five days keeps them approximately so.

We desire to record our indebtedness to Mr. James Krucky, technical assistant in this work, for his painstaking care and fidelity to the highest ideals of exact experimental work.

In the work discussed in this paper no attempt has

been made to keep the flies in aseptic culture as had been done by Loeb and Northrop (*eve. cit.*) and other workers. Our choice in the matter has not been dictated by technical difficulties, which are not great, but has been deliberate. Aseptic life is by no means normal life for *Drosophila*. Normally it is as loaded with a bacterial flora as we are. It was felt that in the beginning it would be well to establish norms of duration of life for normal life conditions. Later we expect to make a special study of duration of life under aseptic conditions.

Duration of life in this work with *Drosophila* is always measured in days, and all of our records relate to duration of adult or imaginal life. No account is taken in any figures of the larval or pupal stages. The reason for this convention is first accuracy and second convenience. It is far more difficult to measure accurately either larval or pupal duration of life than it is imaginal. And from the point of view of these studies nothing significant in principle is lost by dropping these early stages, so far as we have been able to discover, either from the literature or experience with the flies.

MORTALITY CURVES

The most exact and comprehensive manner in which the facts about the duration of life in any organism can be presented is by means of life tables, of the type used for many years by actuaries in their work. The biologically essential features of a life table may be mentioned here briefly for the benefit of biologists not immediately familiar with the development of actuarial science. A complete life table includes, *inter alia*, the following items:

1. The number of individuals surviving up to each of the ages x_0, x_1, etc., out of a given number (1,000 or 10,000 or whatever number one chooses) assumed to have started life together at exactly the same instant of time. These survival frequencies taken together constitute what is technically known as the l_x line of a life table.

2. The number of individuals dying within any short

interval of time, say between x and $x+1$, or $d_x = l_x - l_{x+1}$. These frequencies of death taken together constitute the so-called d_x line of a life table.

3. The death rates at each time (or age) x; *i.e.*, the ratio of the deaths between time x and $x+1$ to the survivors at the time x. These observations together constitute what is known as the q_x line of a life table $q_x = (l_x - l_{x+1})/l_x$.

4. The curtate expectation of life of individuals at a given age x. This is the mean or average after life time of all those individuals alive at age x neglecting fractions of the x interval. These observations together constitute the e_x line of a life table.

$$e_x = \frac{l_{x+1} + l_{x+2} + l_{x+3} + \text{etc.}}{l_x}.$$

These simple definitions state with entirely sufficient accuracy for present purposes the significance of the constants which we shall present. Any one wishing to go more particularly into details of actuarial methods will find a useful elementary introduction in Henderson (22) or Dawson (23).

It is our purpose to present here life tables for four groups of flies, to serve, first, to show the general laws of mortality in *Drosophila* as compared with man, the only other organism for which we have extensive and exact life tables; and second, as a normal base for comparison in experimental work on *Drosophila* to be reported in subsequent papers.

The groups for which complete tables are presented are these:

1. *Long-winged males.* This table includes all our data up to June, 1921, on normal (*i.e.*, not experimentally modified) duration of life of male *Drosphila* individuals at 25° C. belonging to the following stocks (cf. p. 486 *supra*): Old Falmouth, New Falmouth, Sepia and Eagle Point. In these stocks all the individuals have wild type wings, hence the designation "long-winged."

2. *Long-winged females.* The corresponding table to 1, but for females.

3. *Short-winged males.* This table includes all our data up to June, 1921, on the normal duration of life at 25° C. of males belonging to the Quintuple stock. These flies carry the wing mutation vestigial; hence the designation "short-winged."

4. *Short-winged females.* The corresponding table to 3, but for females.

We have tried a number of different plans for the graduation of these tables, and wish to acknowledge gratefully the helpful suggestions of our colleague in the department, Dr. Lowell J. Reed, in connection with this phase of the work. It was first found that a rather satisfactory result could be obtained by fitting a logarithmic parabola of the type

$$y = a + bx + cx^2 + d \log x$$

to the q_x data. Working from this as a basis we finally decided that, as a practical matter, results on the whole most satisfactory could be got by the following type of graduation.

$$\log l_x = e^{a x}(a + bx + cx^2 + dx^3). \qquad \text{(i)}$$

This amounts to asserting that the instantaneous death rate increases with age as a modified logarithmic function of x.

The actual equations for the four calculated l_x lines of Tables II to V inclusive, together with the absolute number of individuals on which the curves are based, are as follows:

Long winged ♂s (4,586 flies):

$$\log l_x = e^{-.00838509x}(3.0041905 - .02937911x + .000140245x^2 - .0000015897x^3). \qquad \text{(ii)}$$

Long winged ♀s (5,426 flies):

$$\log l_x = e^{-.00481563x}(3.0042303 - .01869993x + .000059620x^2 - .0000020438x^3). \qquad \text{(iii)}$$

Short winged ♂s (854 flies):

$$\log l_x = e^{-.05718877x}(3.0085931 - .17931770x + .004010630x^2 - .0000332501x^3). \qquad \text{(iv)}$$

Short winged ♀s (906 flies):

$$\log l_x = e^{-.04601387x}(3.0116555 - .14948615x + .002851219x^2 - .0000210642x^3). \qquad \text{(v)}$$

The plan of arrangement of Tables II to V is as follows: The first column gives the age of the flies in days, starting theoretically from the time of the emergence of the imago from the pupa as zero. Since the flies spend on the average a day in the breeding bottle before they are taken out into the small duration-of-life bottles, and the deaths are not observed for this interval, our distributions as recorded actually start with age 1 instead of age 0. The next two columns give the *observed* deaths and survivors on the basis of a thousand individuals at "birth" (here emergence as imago). The next three columns give the *calculated* (graduated) values deduced from equations (ii) to (v) above; first the l_x line, next the q_x, and finally the e_x, the latter values being of course in days. Owing to the fact that no premium rates are likely to be calculated from these life tables, we have not thought it necessary to keep but one place of decimals in the case of the q_x and e_x lines, and none whatever in the l_x line. Of course, in the computations more decimal places were kept, and these life tables may be regarded as accurate to a considerably higher degree than the figures as here published indicate. But, on the other hand, so far as we can see, the figues here tabled are sufficiently detailed for any use to which they are ever likely to be put.

The l_x lines of Tables II to V are shown graphically in Figs. 1 and 2. The diagrams are plotted to an arithlog grid, the scale of the abscissæ being divided arithmetically, and that of the ordinates logarithmically. Field (24) has shown the advantages of this method of plotting life table l_x lines. He says:

> In the natural-scale diagram the descent of the curve expresses the number of deaths in a year among the survivors to a given age. This is not the usual way of stating death-rates; nor is it a convenient method, since the absolute number of deaths is a joint resultant of two factors which might better be considered separately—the probability of death at the specified age, and the number of persons at that age and subject to that hazard. We are ordinarily more concerned with the probability alone, or, which is much the same

TABLE II

LIFE TABLE FOR DROSOPHILA—LONG-WINGED MALES

Age in Days	Observed		Calculated			Age in Days	Observed		Calculated		
	d_x	l_x	l_x	q_x	e_x		d_x	l_x	l_x	q_x	e_x
1	5	1,000	1,000	9.6	41.0	50	15	363	368	45.8	14.2
2	12	995	990	9.7	40.4	51	20	348	351	47.7	13.8
3	6	983	981	9.7	39.7	52	19	328	334	49.6	13.5
4	13	977	971	9.7	39.1	53	22	309	318	51.6	13.1
5	10	964	962	9.9	38.5	54	16	287	301	53.7	12.8
6	10	954	952	10.0	37.9	55	13	271	285	55.7	12.4
7	15	944	943	10.1	37.2	56	19	258	269	57.9	12.1
8	9	929	933	10.3	36.6	57	12	239	254	60.2	11.8
9	9	920	924	10.4	36.0	58	19	227	238	62.5	11.5
10	9	911	914	10.6	35.4	59	13	208	224	64.8	11.2
11	12	902	904	10.8	34.7	60	12	195	209	67.3	10.9
12	8	890	895	11.0	34.1	61	18	183	195	69.8	10.6
13	8	882	885	11.3	33.5	62	8	165	181	72.4	10.3
14	11	874	875	11.6	32.8	63	13	157	168	75.2	10.1
15	8	863	865	12.0	32.2	64	12	144	156	77.9	9.8
16	14	855	854	12.2	31.6	65	13	132	143	80.8	9.5
17	8	841	844	12.6	31.0	66	14	119	132	83.6	9.3
18	13	833	833	13.0	30.3	67	7	105	121	86.7	9.0
19	10	820	822	13.4	29.7	68	8	98	110	89.8	8.8
20	11	810	811	13.9	29.1	69	5	90	100	92.9	8.6
21	16	799	800	14.4	28.5	70	8	85	91	96.1	8.4
22	6	783	789	14.9	27.9	71	5	77	82	99.6	8.1
23	13	777	777	15.4	27.3	72	7	72	74	102.9	7.9
24	11	764	765	16.0	26.7	73	8	65	67	106.4	7.7
25	11	753	753	16.6	26.2	74	3	57	59	110.0	7.5
26	10	742	740	17.3	25.6	75	9	54	53	113.8	7.3
27	10	732	727	17.9	25.0	76	2	45	47	117.3	7.1
28	14	722	714	18.7	24.4	77	8	43	41	121.5	6.9
29	11	708	701	19.4	23.9	78	4	35	36	125.4	6.8
30	15	697	687	20.2	23.3	79	4	31	32	129.2	6.6
31	13	682	673	21.1	22.8	80	3	27	28	133.6	6.4
32	11	669	659	21.9	22.3	81	3	24	24	137.5	6.3
33	15	658	645	22.9	21.8	82	4	21	21	142.0	6.1
34	7	643	630	23.8	21.2	83	2	17	18	146.4	5.9
35	18	636	615	24.8	20.7	84	1	15	15	151.0	5.8
36	15	618	600	25.8	20.2	85	2	14	13	156.2	5.7
37	19	603	584	26.9	19.7	86	2	12	11	160.2	5.5
38	13	584	569	28.1	19.3	87	1	10	9	164.5	5.4
39	22	571	553	29.3	18.8	88	2	9	8	170.6	5.2
40	15	549	536	30.5	18.3	89	2	7	6	175.6	5.1
41	13	534	520	31.8	17.9	90	0	5	5	180.4	5.0
42	23	521	503	33.2	17.4	91	1	5	4	185.0	4.9
43	19	498	487	34.5	17.0	92	1	4	3	189.7	4.8
44	22	479	470	36.0	16.6	93	1	3	3	196.3	4.6
45	18	457	453	37.5	16.1	94	0	2	2	201.8	4.5
46	22	439	436	39.0	15.7	95	1	2	2	207.5	4.4
47	19	417	419	40.7	15.3	96	0	1	1	212.8	4.3
48	15	398	402	42.3	14.9	97	0	1	1	218.3	4.2
49	20	383	385	44.0	14.6						

TABLE III

Life Table for Drosophila—Long-winged Females

Age in Days	Observed		Calculated			Age in Days	Observed		Calculated		
	d_x	l_x	l_x	q_x	e_x		d_x	l_x	l_x	q_x	e_x
1	5	1,000	1,000	9.7	38.8	46	19	386	384	46.4	14.3
2	14	995	990	9.7	38.1	47	19	367	367	48.3	13.9
3	10	981	981	9.8	37.5	48	13	348	349	50.2	13.6
4	13	971	971	9.9	36.9	49	23	335	331	52.1	13.2
5	12	958	961	10.0	36.2	50	16	312	314	54.1	12.9
6	10	946	952	10.1	35.6	51	20	296	297	56.1	12.6
7	13	936	942	10.3	34.9	52	15	276	281	58.2	12.3
8	8	923	932	10.5	34.3	53	15	261	264	60.4	12.0
9	11	915	923	10.8	33.6	54	17	246	248	62.6	11.7
10	13	904	913	11.0	33.0	55	11	229	233	64.9	11.4
11	12	891	903	11.4	32.4	56	17	218	218	67.2	11.1
12	6	879	892	11.8	31.7	57	13	201	203	69.6	10.8
13	9	873	882	12.1	31.1	58	12	188	189	72.0	10.5
14	12	864	871	12.4	30.4	59	15	176	175	74.5	10.3
15	8	852	861	12.9	29.8	60	12	161	162	77.1	10.0
16	12	844	849	13.3	29.2	61	11	149	150	79.8	9.8
17	9	832	838	13.9	28.6	62	11	138	138	82.4	9.6
18	11	823	827	14.4	28.0	63	9	127	126	85.1	9.3
19	17	812	815	15.0	27.4	64	9	118	116	87.9	9.1
20	10	795	802	15.6	26.8	65	12	109	105	90.8	8.9
21	22	785	790	16.2	26.2	66	10	97	96	93.8	8.7
22	10	763	777	16.9	25.6	67	7	87	87	96.7	8.5
23	16	753	764	17.6	25.0	68	11	80	78	99.8	8.3
24	14	737	751	18.4	24.4	69	5	69	71	102.9	8.1
25	10	723	737	19.2	23.9	70	10	64	63	106.2	7.9
26	12	713	723	20.1	23.3	71	3	54	57	109.2	7.7
27	14	701	708	20.9	22.8	72	7	51	50	112.5	7.5
28	18	687	693	21.9	22.2	73	3	44	45	115.9	7.3
29	16	669	678	22.9	21.7	74	4	41	40	119.4	7.2
30	16	653	663	23.9	21.2	75	5	37	35	123.0	7.0
31	12	637	647	24.9	20.7	76	1	32	31	126.2	6.8
32	13	625	631	26.0	20.2	77	7	31	27	130.2	6.7
33	15	612	614	27.2	19.7	78	4	24	23	133.4	6.5
34	14	597	597	28.4	19.2	79	1	20	20	137.5	6.4
35	18	583	581	29.6	18.8	80	3	19	17	141.6	6.2
36	17	565	563	30.9	18.3	81	2	16	15	144.9	6.1
37	16	548	546	32.2	17.9	82	4	14	13	149.0	6.0
38	17	532	528	33.6	17.4	83	1	10	11	153.0	5.8
39	19	515	511	35.0	17.0	84	1	9	9	157.8	5.7
40	17	496	493	36.5	16.6	85	1	8	8	161.5	5.6
41	15	479	475	38.0	16.2	86	2	7	6	164.9	5.4
42	13	464	457	39.7	15.8	87	0	5	5	169.7	5.3
43	15	441	439	41.2	15.4	88	1	5	4	173.3	5.2
44	21	426	420	42.9	15.0	89	1	4	4	180.1	5.1
45	19	405	402	44.6	14.6	90	0	3	3	183.6	5.0
						91	1	3	2	188.7	4.9
						92	0	2	2	192.1	4.8
						93	1	2	2	195.1	4.7
						94	0	1	1	204.5	4.6
						95	0	0	1	209.5	4.5

TABLE IV

LIFE TABLE FOR DROSOPHILA—SHORT-WINGED MALES

Age in Days	Observed		Calculated			Age in Days	Observed		Calculated		
	d_x	l_x	l_x	q_x	e_x		d_x	l_x	l_x	q_x	e_x
1.....	6	1,000	1,000	25.6	14.2	24.....	16	151	155	107.5	8.0
2.....	27	994	974	31.5	13.6	25.....	13	135	139	107.3	7.8
3.....	30	967	944	37.5	13.0	26.....	11	122	124	107.1	7.7
4.....	34	937	908	43.3	12.4	27.....	6	111	111	107.2	7.5
5.....	38	903	869	48.9	12.0	28.....	20	105	99	107.3	7.2
6.....	36	865	826	54.6	11.5	29.....	8	85	88	107.9	7.0
7.....	85	829	781	60.0	11.1	30.....	7	77	79	109.2	6.7
8.....	66	744	734	65.5	10.8	31.....	13	70	70	111.1	6.4
9.....	55	678	686	70.4	10.5	32.....	7	57	62	114.5	6.1
10.....	52	623	638	75.2	10.2	33.....	9	50	55	119.3	5.8
11.....	44	571	590	79.8	9.9	34.....	5	41	49	126.2	5.4
12.....	48	527	543	84.0	9.7	35.....	4	36	42	135.4	5.0
13.....	21	479	497	87.9	9.5	36.....	6	32	37	147.4	4.7
14.....	49	458	454	91.5	9.3	37.....	4	26	31	162.7	4.3
15.....	53	409	412	94.8	9.1	38.....	1	22	26	182.2	3.9
16.....	43	356	373	97.7	9.0	39.....	6	21	21	206.2	3.6
17.....	24	313	337	100.2	8.9	40.....	2	15	17	234.7	3.3
18.....	28	289	303	102.1	8.7	41.....	4	13	13	268.6	3.0
19.....	22	261	272	104.1	8.6	42.....	1	9	10	308.0	2.7
20.....	19	239	244	105.3	8.5	43.....	2	8	7	352.9	2.4
21.....	24	220	218	106.3	8.4	44.....	5	6	4	403.0	2.2
22.....	17	196	195	106.8	8.3	45.....	0	1	3	457.9	2.0
23.....	28	179	174	107.2	8.1	46.....	0	1	1	516.0	1.8

thing, with the proportion of those persons of given age who die in the course of a year. Precisely this relative mortality rate determines the slope of the curve in the logarithmic figure, for here, as always, a given distance on the logarithmic scale denotes a certain proportion of change. Hence the more steeply the logarithmic curve descends, the higher is the relative mortality which it indicates. Hence, too, it is possible to provide a key to the diagram in the form of standard sample slopes and corresponding numerical death-rates, which hold true for all parts of the curve.

From these tables and the diagrams, the following points are to be noted:

1. It is obvious that the laws of mortality are fundamentally similar in *Drosophila* to what they are in man, with the one striking and outstanding difference that since in the case of the *Drosophila* life tables we are deal-

TABLE V

LIFE TABLE FOR DROSOPHILA—SHORT-WINGED FEMALES

Age in Days	Observed		Calculated			Age in Days	Observed		Calculated		
	d_x	l_x	l_x	q_x	e_x		d_x	l_x	l_x	q_x	e_x
1	10	1,000	1,000	30.2	15.8	27	13	177	165	93.1	8.5
2	26	990	970	33.9	15.2	28	18	164	149	94.6	8.3
3	37	964	937	37.6	14.7	29	11	146	135	96.1	8.0
4	31	927	902	41.2	14.3	30	20	135	122	98.1	7.8
5	47	896	865	44.7	13.9	31	15	115	110	100.2	7.5
6	27	849	826	48.1	13.5	32	15	100	99	102.8	7.2
7	50	822	786	51.5	13.1	33	8	85	89	106.3	6.9
8	78	772	746	54.7	12.7	34	5	77	80	110.0	6.6
9	45	694	705	57.9	12.4	35	7	72	71	114.5	6.3
10	63	649	664	60.9	12.1	36	10	65	63	120.2	6.0
11	34	586	624	63.8	11.8	37	6	55	55	127.1	5.7
12	35	552	584	66.6	11.6	38	7	49	48	134.8	5.4
13	25	517	545	69.2	11.3	39	8	42	42	144.4	5.1
14	39	492	507	71.7	11.1	40	8	34	36	155.8	4.8
15	31	443	471	74.1	10.9	41	8	26	30	168.4	4.5
16	30	412	436	76.2	10.7	42	3	18	25	183.8	4.2
17	28	382	403	78.3	10.5	43	1	15	20	201.3	3.9
18	28	354	371	80.2	10.3	44	3	14	16	221.2	3.6
19	25	326	341	82.0	10.1	45	1	11	13	243.8	3.3
20	14	301	313	83.6	9.9	46	0	10	10	269.9	3.1
21	20	287	287	85.1	9.7	47	2	10	7	297.0	2.8
22	14	267	263	86.6	9.5	48	0	8	5	328.9	2.6
23	21	253	240	87.9	9.3	49	2	8	3	363.3	2.4
24	21	232	219	89.2	9.1	50	3	6	2	401.0	2.2
25	16	211	199	90.4	8.9	51	1	3	1	457.2	2.0
26	18	195	181	91.7	8.7						

ing only with the duration of imaginal life, the important infant and early childhood mortality component of the human d_x line is entirely omitted. With this difference in mind, it is apparent that the remainder of the l_x curve for the flies is essentially similar to any human l_x curve. Further on, we shall make a more detailed comparison between *Drosophila* and human curves.

2. There is evidently a fundamental and marked difference between the long-winged and short-winged groups in respect of the duration of life. This difference is somewhat more marked in the case of the males (Fig. 1) than in the females, but it is sufficiently definite and clear-cut

FIG. 1. Diagram showing the observed and graduated l_x poirts for long-winged and short-winged males, respectively. The small circles are the observations and the smooth lines the fitted curves from equations (ii) and (iv). In order not to overcrowd the diagram, only every second observation is shown.

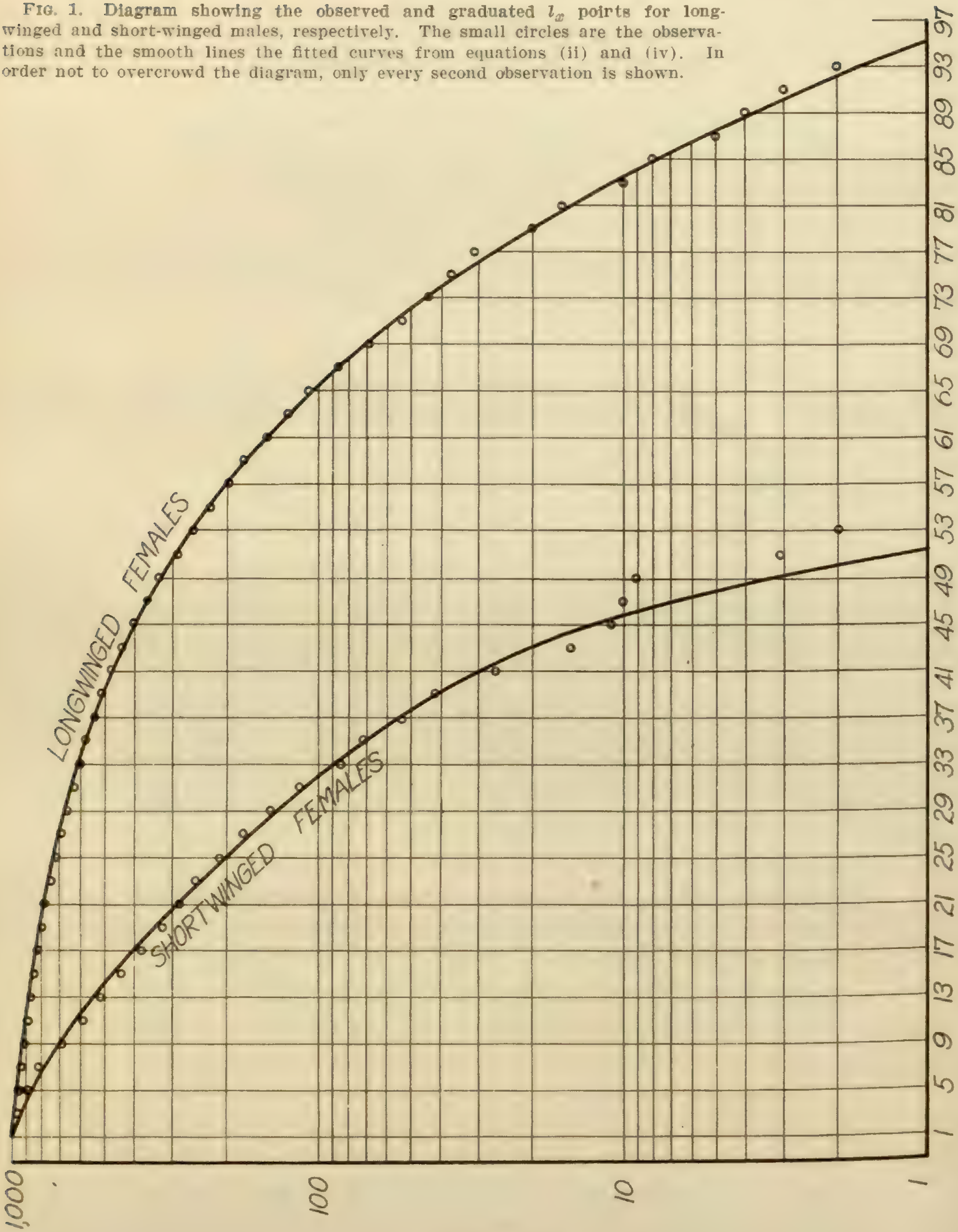

FIG. 2. Diagram showing the observed and graduated l_x points for long-winged and short-winged females, respectively. The small circles are the observations and the smooth lines the fitted curves from equations (iii) and (v). In order not to overcrowd the diagram, only every second observation is shown.

in both cases. Broadly speaking, the wild type long-winged flies have from two to three times as great an expectation of life at any age as do the flies of the Quintuple stock. Since all of these flies lived under substantially identical environmental conditions, as has been set forth earlier, it follows that the basis of the great difference in expectation of life between these two groups, as exemplified in Figs. 1 and 2, *is hereditary and not environmental.*

3. It is apparent that on the whole the graduations given by equations of the type of (i) are very satisfactory, and as good as could reasonably be expected on experience bases of the magnitude of those here dealt with. Undoubtedly the curves would be slightly more smooth if we had larger experience, especially in Tables IV and V, where we are dealing with less than a thousand flies in each case, but in the main the curves fit the observations very well.

4. The death rates (q_x) generally increase steadily with advancing age. An exception to this rule is the slight dip between ages 25 28 in the short-winged ♂ table.

In Figs. 3 and 4 the *Drosophila* l_x lines are compared with the human l_x line taken from Glover's (25) 1910 U. S. Life Tables. In order to make a just comparison, the human l_x line is displaced to the left in the diagrams until age fifteen of human life coincides with age one of the fly curve. This drops out the infant and childhood mortality component of the human curve. It will be understood that in the present instance, this is a somewhat arbitrary and purely graphic procedure. Whether the point which *exactly* corresponds on the human curve to the beginning of the *Drosophila* imago curve is exactly 15 years or 13 or 14, or some other near-by value, is a matter for further research. In a broad way, however, it is clear that the two lines must be taken to correspond at something like this point in the human curve.

From these diagrams, it is apparent that, after leaving out the infant mortality component of the human curve,

the essential difference between the human and *Drosophila* l_x curves is that, up to what may be designated as the end of the middle life portion (and even into the early part of the old age portion), human beings have a *relatively* better expectation of life than does *Drosophila*. On the other hand, in the extreme old age portion of the curve, the *Drosophila* expectation of life is relatively

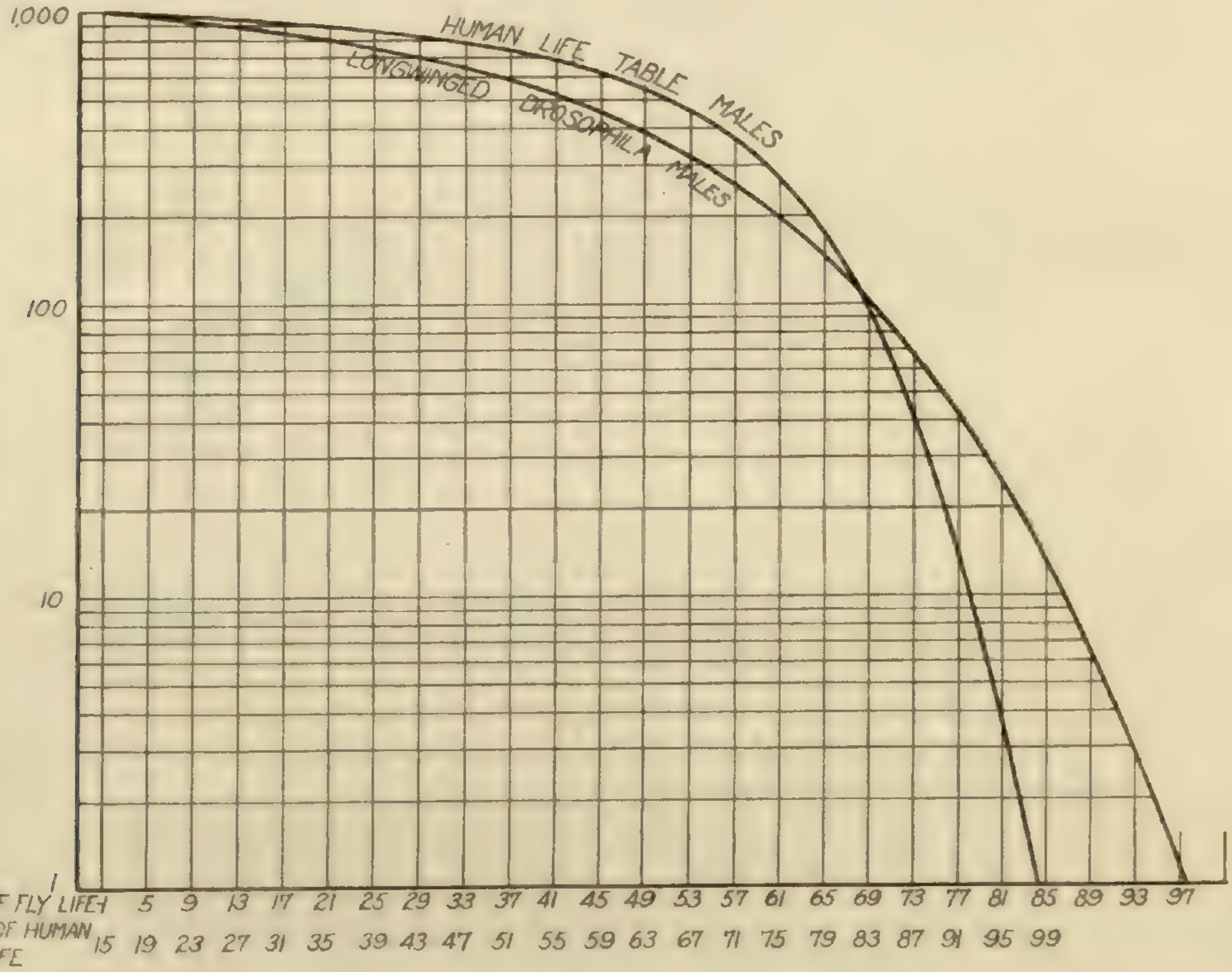

FIG. 3. A comparison, of human l_x and *Drosophila* l_x lines for males.

better than the human. The result then is as though some external power had seized the *Drosophila* l_x line at about the middle of its course and bent it to a sharper angle in that region, stretching it at that point upward and to the right and by this process converted it into the human curve. Suppose one of the *Drosophila* l_x lines, as shown in Fig 3, to be a thin, flexible whalebone rod, possessing mass. Then move a point on that rod standing say just above the final *A* in "*Drosophila*" in Fig. 3, up to a point where it exactly coincides with the human life table curve. Then the whole of the rest of the *Drosophila* curve would fall into about the same posi-

tion as is occupied now by the human life table curve. Put in another way, what appears to have happened is that, as compared with *Drosophila,* more human beings are able to live through middle life, but at the expense of those who, if the mortality law was the same as in *Drosophila,* would live to extremely advanced old ages. As a matter purely of speculation in the present stage of our knowledge, it may be suggested that the *Drosophila*

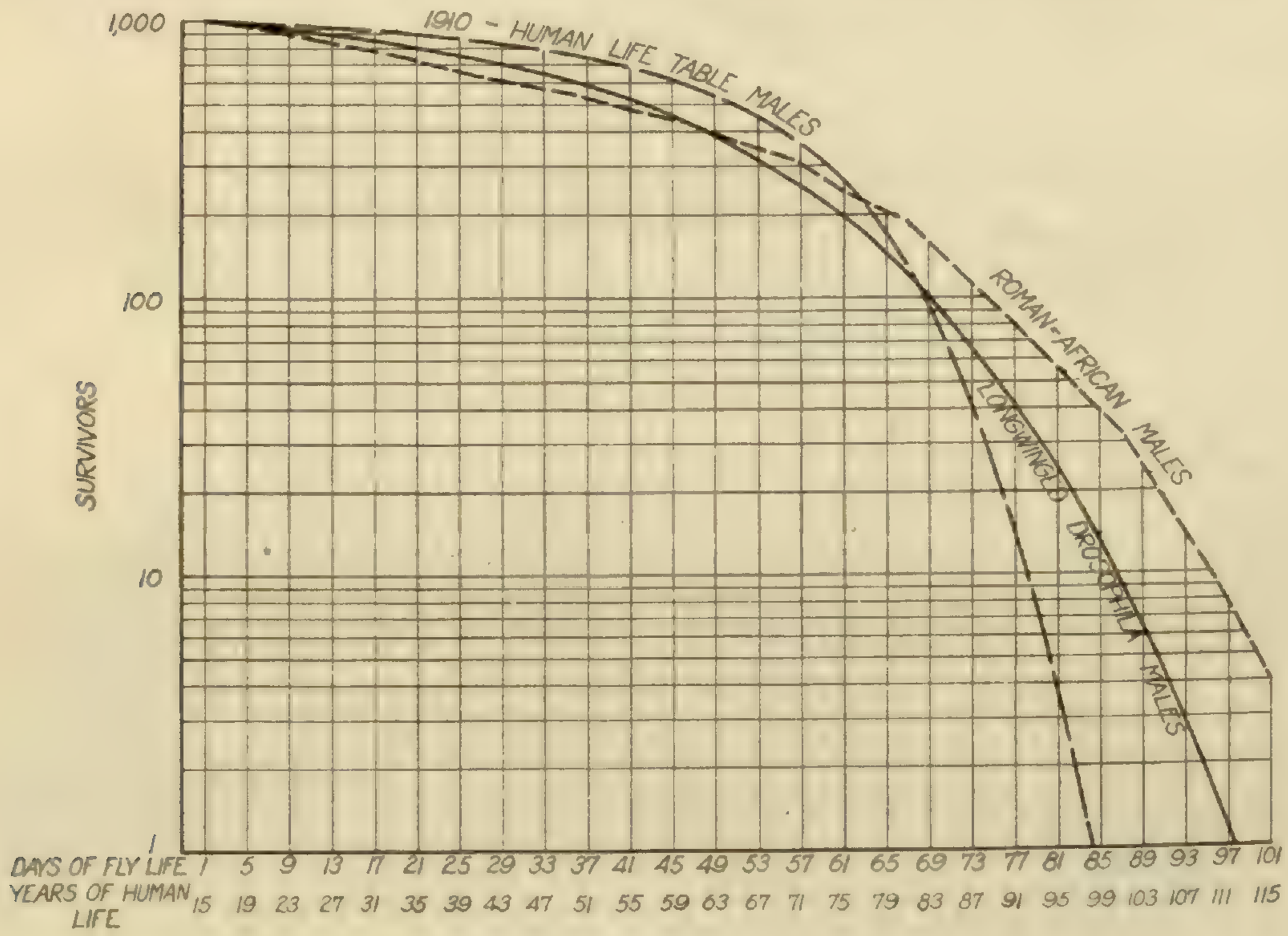

FIG. 4. A comparison of human l_x and *Drosophila* l_x lines for females.

l_x curves represent more nearly the normal, fundamental, biological law of mortality, and that the human curve has been warped from this form as a result of those activities which may be comprised under the terms public health and sanitation. It is to be understood that at present we offer this merely as a suggestion and in no way as a settled conclusion. It is, however, clear that the effect which we should expect these activities to have upon the form of the l_x line is exactly of the sort which makes the human curve different from the *Drosophila* in fact.

In this connection Fig. 5 is of interest and significance.

It compares, for males, two human l_x lines nearly 2,000 years apart in point of time, with the long-winged *Drosophila* l_x line of Table II. The two human lines are (*a*) Glover's, 1910 U. S. table (as in Fig. 3), and (*b*) Macdonell's (26) observed l_x line from the population of Roman provinces in Africa at about the beginning of the Christian era, his data having been taken from gravestone inscriptions. We calculated the l_x line here plotted from Macdonell's tabled d_x data, determining an l_x point at each quinquennium. This smooths the Roman-African figures somewhat, and makes the l_x line so determined lie very slightly higher all along its course than would be the case if we used a more elaborate and exact mathematical procedure. The error, however, is so small that it would scarcely be discernible in the scale at which Fig. 5 is reproduced.

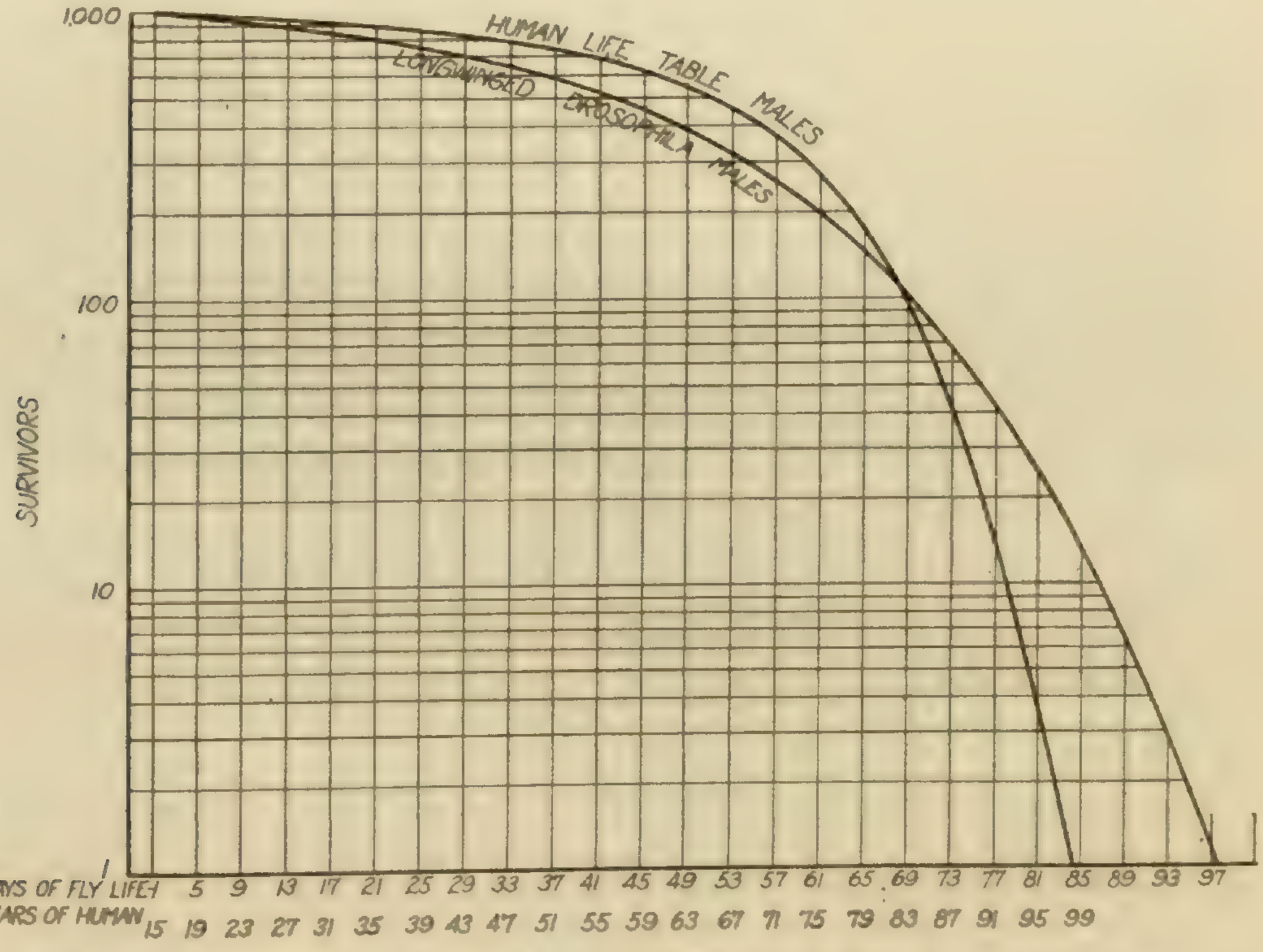

FIG. 5. A comparison of *Drosophila* mortality with human mortality at two periods (*a*) early in the Christian era, and (*b*) in 1910.

It is at once apparent from Fig. 5 that the *Drosophila* survival curve runs, in general throughout its course, *between* the curve for human beings 18 or 19 centuries

ago and that for the present time. As compared with Glover's 1910 U. S. tables, the Roman population of Africa at the dawn of our era was, in respect of the course of its mortality, even more *Drosophila*-like than *Drosophila* itself! Now in Roman Africa there was relatively little of what we now understand as sanitation, hygiene, and preventive medicine. Men lived to old age, if they did, by virtue mainly of the strength of their innate constitutions, and their good luck in avoiding fatal accidents. At the present time hospitalization, the science and art of medicine and public health, and general sanitation keep many persons alive well into middle age who would in those days have died much earlier because of a lack of constitutional ruggedness. Altogether the data of Fig. 5 seem highly significant in relation to the hypothesis suggested above as to the reason for the difference between *Drosophila* and man in respect of mortality curves.

ACCIDENTAL DEATHS

The tacit assumption in all the foregoing is that each of the 11,772 flies comprised in the four life tables died a natural death, and that the time of death (or duration of life) was in each case determined fundamentally by internal factors, since the environment was substantially a constant for all.

For certainly more than 98 per cent. of the flies this assumption is unquestionably true. But in view of the possibility that some few of the flies might be dying accidental deaths by drowning in the moisture, which sometimes collects on the surface of the food, it was thought worth while to attempt to prevent the collection of moisture in some of the bottles and compare the duration of life of flies kept in such bottles with that of flies kept under the ordinary conditions. Accordingly, bottles of food were prepared by putting discs of several layers of a very absorbent paper, Zorbik, in the bottom of the bottle and then pouring in the food and letting it solidify

on as steep a slant as possible, so that any moisture formed would drain down and be absorbed by the paper in the bottom of the bottle. Flies of generation 5 from four different lines were used in the experiment, two normal wild type lines, one of Old Falmouth stock and one of New Falmouth stock, and two lines of Quintuple stock. Flies were taken out from stock bottles of these lines as they emerged every day, beginning March 18, 1921, and continuing through April 4, putting hatches of alternate days in the specially prepared bottles and the other hatches in ordinary bottles. The flies put into the specially prepared bottles were of course kept throughout their lives in such bottles, and the controls in ordinary bottles. Table VI shows the l_x lines of the four groups of flies—long-winged with paper and slant food, long-winged without paper and food surface horizontal, short-winged with paper and slant food, short-winged without paper and food surface horizontal. Distributions have been made for the four lines separately, and for the sexes separately, but since they all show the same results the separate distributions are not given.

The data of Table VI are shown graphically in Fig. 6.

It is evident that there is no definite or marked difference between the slant food group and the other. Such differences as do appear between the l_x lines in the two cases are only of the order of magnitude which might readily appear in random sampling. This is indicated in another way by the data of Table VII.

In the case of the short-winged flies the difference in the mean is plainly not significant. In the case of the long-winged flies the difference is 2.96 times its probable error. One would expect a difference as great as this or greater to occur from chance alone only 4 to 5 times in every 100 trials, so that the difference is here getting on towards the magnitude where it must be regarded as certainly significant on purely statistical grounds. But the difference is in favor of the horizontal food without drainage, and *against* the food with drainage.

TABLE VI

SURVIVAL DISTRIBUTION OF FLIES UNDER DIFFERENT CONDITIONS AS TO SURFACE MOISTURE ON FOOD

Age	Short-winged Flies		Long-winged Flies	
	With Paper and Slant Food	Without Paper. Food Surface Horizontal	With Paper and Slant Food	Without Paper. Food Surface Horizontal
1	1,000	1,000	1,000	1,000
3	983	1,000	—	—
6	913	951	996	1,000
9	804	857	—	—
12	712	774	974	982
15	651	660	—	—
18	556	498	898	912
21	469	442	—	—
24	346	355	834	832
27	257	302	—	—
30	182	234	796	799
33	115	147	—	—
36	84	83	675	770
39	59	38	—	—
42	22	15	547	661
45	17	4	—	—
48	14	0	408	482
51	8	—	—	—
54	3	—	294	314
57	0	—	—	—
60	—	—	204	230
63	—	—	—	—
66	—	—	94	157
69	—	—	—	—
72	—	—	34	120
75	—	—	—	—
78	—	—	23	51
81	—	—	—	—
84	—	—	15	7
87	—	—	—	—
90	—	—	4	0
Absolute number of flies.	(265)	(274)	(358)	(265)

On the whole it seems perfectly clear that these experiments give no justification for going to the considerably greater trouble of preparing this food so that there is drainage from its surface. As a matter of fact the drainage of moisture is never entirely complete even with

TABLE VII

MEAN DURATIONS OF LIFE CALCULATED FROM THE DATA OF TABLE VI

	Mean Duration of Life		Difference of Means	Diff. / P.E. Diff.
	With Paper and Slant Food	Without Paper and Slant Food		
Long-winged	43.80 ± .73	46.91 ± .75	3.10 ± 1.05	2.96
Short-winged	20.10 ± .40	20.58 ± .42	.48 ± .58	.83

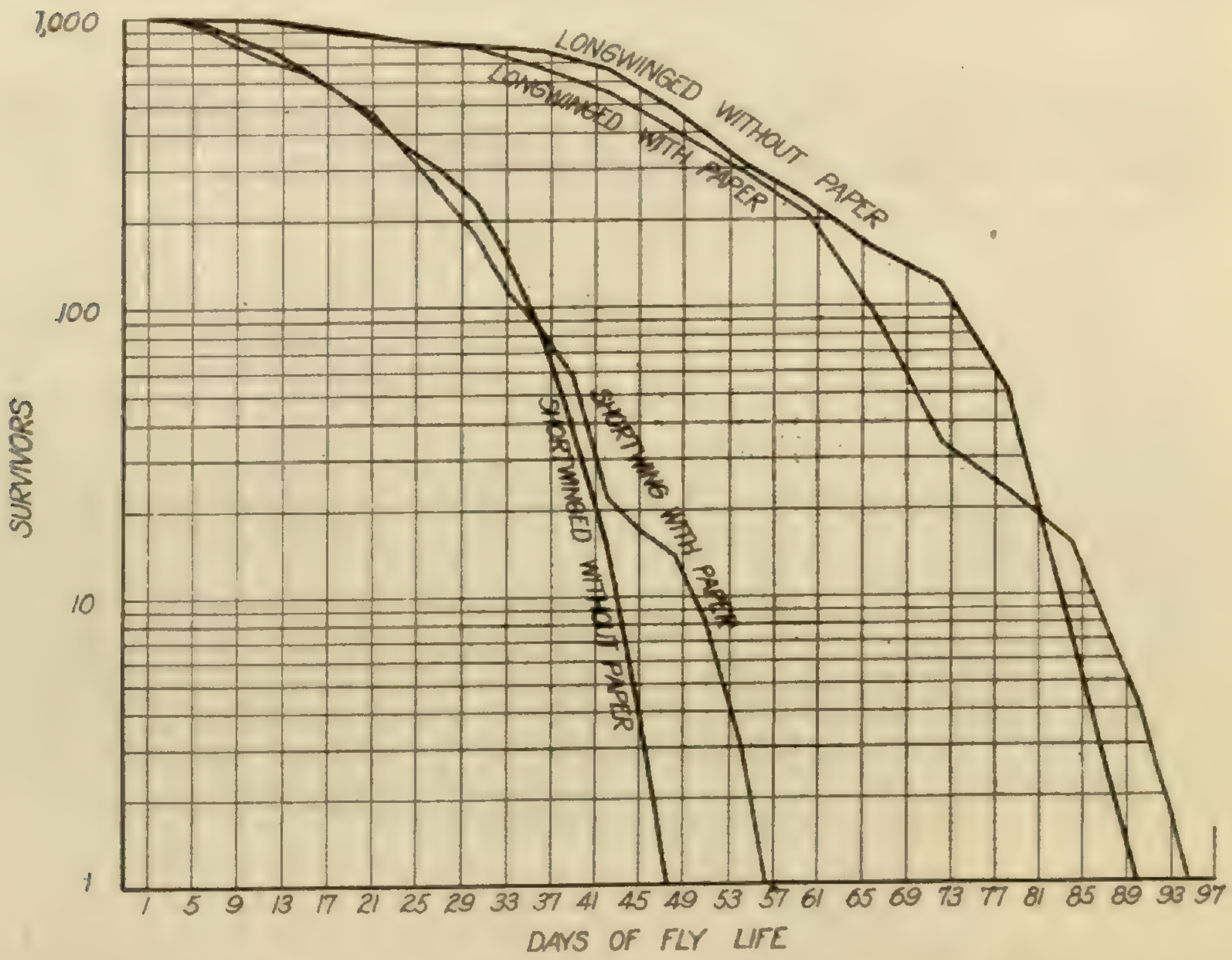

FIG. 6. Comparing survival (l_x) lines under different food conditions explained in text.

the slant food and absorbing paper. Small drops still cling in some cases to the agar, and a fly might drown in such a drop just as well as in a similar drop on a horizontal surface. The important point is that this experiment confirms our general experience in this work, namely, that accidental deaths occur so extremely rarely under our conditions that they do not appreciably affect the results.

SUMMARY

This paper is the first in a series of experimental studies on the factors influencing the duration of life in *Drosophila melanogaster*. An account of the experimental technique used in these duration of life studies is presented. Four complete life tables for *Drosophila* are given, and it is shown that this organism follows quantitatively the same general law in respect of the distribution of its mortality as does man. As this work deals only with the duration of imaginal life in *Drosophila* there is no component in the life tables corresponding to the mortality of infancy and childhood in man. It is shown that there are wide differences in duration of life in different stocks of *Drosophila*, and that the basis of these differences is hereditary and not environmental. The *Drosophila* survival line of the life table (l_x) runs in general throughout its course between human survival lines of (*a*) the present time, and (*b*) about the beginning of the Christian era (Macdonell's data from Roman Africa), the curves being superposed on the basis of the omission of the human mortality of infancy and childhood.

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INHERITANCE OF CANCER IN MICE

DR. LEO LOEB

THE DEPARTMENT OF COMPARATIVE PATHOLOGY, WASHINGTON UNIVERSITY SCHOOL OF MEDICINE, ST. LOUIS

IN the following we shall give a summary of our investigations into the part heredity plays in the origin of cancer. Our interest in this problem dates back a considerable number of years. In 1899, in conjunction with Dr. Jobson, we made our first observations on the endemic occurrence of cancer at the Stockyards in Chicago. At that time we found cattle coming from a certain ranch in Wyoming especially prone to have cancer at the inner canthus of the eye (1). A few years later we observed an endemic occurrence of sarcoma of the thyroid in a family of rats. In this case we pointed out that the circumstances under which these sarcomata originated pointed to a hereditary condition rather than to an infection. At that time (1904)[1] we referred to the desirability of investigating experimentally this endemic occurrence of cancer (2) and we had particularly in mind an analysis of the etiological factor in breeding establishments of mice or rats. Such an opportunity presented itself a few years later in the breeding establishment of Miss Lathrop in Granby, Mass. A preliminary investigation here revealed the fact that cancer occurred with much greater frequency in certain inbred strains than in others, and that no indications whatever could be found of cage infection or direct infection from animal to animal. We concluded in 1907 on the basis of these observations that there existed a hereditary predisposition which was responsible for the endemic occurrence of cancer (3). At that time we planned further much more extensive experiments on the mode of hereditary transmission of cancer in following isolated families and strains of mice through several generations and in study-

[1] A paper presented in abstract before the II Intern. Congress of Eugenics, New York.

ing the effect of hybridization on the cancer rate. This was made possible to us in 1910 through the interest which Miss Lathrop, who bred a very extensive stock of mice, took in these plans and with her cooperation we carried out these breeding experiments from 1910 on through a considerable number of years. In the following year we published the record of a cancer family of mice which we had observed through several generations (4). Altogether we carried out observations on approximately 12,000 female mice reaching the cancer age which we followed throughout the whole period of their life and which were observed through successive generations. Many of them were used for hybridization.

In the meantime E. E. Tyzzer had published in 1907 and subsequently studies of the inheritance of cancer in mice (5). This author found indications that heredity may play a part in the etiology of animal cancer. Somewhat later Murray undertook similar studies in which he used methods similar to those previously employed in the study of human cancer. This author compared the frequency of cancer among individuals whose mother or grandmother had cancer on the one hand, and among those in whom cancer had not been observed in the direct ancestry but may have occurred in the great grandparents (6). Murray found on the average about 20 per cent. of cancerous mice among those whose direct ancestors had suffered from cancer and 11 per cent. among those whose direct ancestry had been free from cancer. In certain age classes the cancer rate of both kinds of mice showed only a very slight difference and in one age class the cancer rate was even higher in those whose direct ancestry had not been affected by cancer.

These results as well as our previous studies and some occasional observations of Albrecht and Hecht and of Spronk and a few others made very probable the significance of heredity in the etiology of cancer.

Nevertheless there were opposing views such as those of Borrel and others who referred the endemic occurrence

of cancer not to heredity, but to infection, and as late as 1910 Bashford expressed the opinion that heredity has no significance in the causation of cancer.

We believe that our investigations which were carried out with the cooperation of Miss Lathrop established the importance of heredity in the etiology of cancer beyond doubt, and they point more accurately to the mode of inheritance and the interaction between heredity and other factors (7). Subsequently Miss Maud Slye began to use a large stock of mice which she had collected for biological purposes for a similar study of heredity of cancer in mice (8). While in general her conclusions as to the significance of heredity in cancer agree with ours and are thus confirmatory of ours, she has extended her researches in various other ways and has made valuable contributions. In regard to certain questions our conclusions differ. To those we shall have occasion to refer in the following pages.

The following is a summary of our main conclusions:

1. The cancer rate of each strain of family is a definite characteristic of this strain and is transmitted by heredity to successive generations. The differences in the tumor rate in various strains are very pronounced; the tumor rate may vary between zero in certain strains and almost 100 per cent. in others. All intergrades may be found. To cite a few examples of tumor rates of various strains: English 67.6 per cent., European + I daughter of No. 10 72 per cent., 344 + 328 = 79 per cent., London 28 per cent., No. 8 27.5 per cent., 8 + German 34 per cent., Cream 5.9 per cent., European 9 per cent., German + 8 0 per cent. (344 + Black Cream) + Cream 0 per cent.

While these strains represent composites, they are on the whole in so far homogeneous, as in the large majority of cases substrains showed similar tumor rates. Thus in the case of the English and Cream, for instance, numerous substrains showed the typical tumor rates. In a number of cases individual families were separated and followed, and on the whole their tumor rate agreed very

well with that of the main strain. Certain deviations must of course be expected in the case of relatively very small numbers of individuals obtained in case of an individual family or small substrains, and yet quite frequently even small substrains or families agree in their tumor rate with the main strain.

We may give some examples of the comparative tumor rates of the main strains and of the substrains: The strain $8\frac{1}{2} + 328$ had a tumor rate of 56.4 per cent. There was among them a family No. 1075 consisting of ten females reaching an age which permitted inclusion in these records. They had a tumor rate of 60 per cent.; another family of this strain (No. 1,113), consisting of sixteen such females, had a tumor rate of 88 per cent. Family 782*a* (22 females) had a tumor rate of 68 per cent. Among the substrain English Sable which had in the corresponding generation an average tumor rate of 75 per cent., there was a family No. 437 (27 females) with a tumor rate of 89 per cent. The same correspondence is found in others and among them low rate strains. In certain cases, however, certain substrains of families can be split off which differ in their tumor rate. Thus at an early period of inbreeding there were split off from the English strain two substrains with low rates: English Silver and English Silver Fawn, with tumor rates of 8 per cent. and 12 per cent., respectively.

From the strain London (tumor rate 28 per cent.) two families were branched off which showed very different rates: London Blue and White (31 females) 55 per cent., and Family 481 (25 females) 0 per cent. But these are not the usual occurrences. A correspondence between main strains, substrains and families is the usual finding.

We see then that all kinds of intergrades in the tumor rates occur in different strains, substrains and families and that these are on the whole constant and characteristic of strains and families.

2. These differences in rate persisted through successive generations in the majority of our strains with a sur-

prising regularity. Thus for instance, in the strain London, the earlier generations (120 female mice) showed a tumor incidence of 27 per cent., in the intermediate generation (61 females) the figure was 38 per cent. and in the later generation (197 female mice) 28 per cent. Similar conditions were found in a number of our strains. In certain strains, however, variations in the tumor rate did occur. While some variations may of course be expected, in case the number of mice considered is very small, there occurred in addition changes which can not be attributed to this factor.

In the majority of cases in which these latter changes did occur in our stock, they consisted in a decrease in the tumor rate in later generations; in a few cases only there occurred an increase in the tumor rate. These changes were in all probability due to two factors: (*a*) In certain families and strains as a result of long continued inbreeding a gradual decrease in fertility and vigor occurred. Associated with this change was in certain cases a noticeable decrease in the tumor rate. Especially in the strain No. 8 there seemed to be a connection between loss in resistance to disease and fertility and the decrease in the tumor rate. This strain was inbred for seventeen generations and the changes in the tumor rate seemed to occur step by step in correspondence with the progress in inbreeding. Under those conditions the connection between inbreeding and change in the tumor rate appears the most probable explanation, although it can not be considered as definitely proven as yet.

(*b*) Various factors caused a selection to take place within the strain; certain families died out, while others, which happened to be more resistant to a certain disease, survived, propagated and thus gained a preponderance. These surviving families differed sometimes in appearance, or in vigor, in the behavior towards certain inoculable tumors. Such changes were accompanied in certain cases by a change in the tumor rate. In the majority of our cases a decrease occurred; in a few cases an increase;

but even in such cases the increase was moderate; there was never observed among our material a sudden transition from a low to a high rate tumor strain. The increase as well as the decrease in the tumor rate was caused by the same factor; whether one or the other should prevail depends more or less on chance, and in different material the number of strains showing the one or the other variation may be expected to differ. It has been maintained that in strains which have been inbred for a long period of time and in which a decrease in fertility occurred as the result of the inbreeding, development of cancers takes the place of the lost fertility. In inbreeding cancer replaces reproduction, as it has been expressed by Maud Slye. In our material such a substitution did not take place; in inbreeding mice vanishing fertility was not replaced by the development of cancer under ordinary conditions. Inbreeding does not lead to an increased cancer rate.

3. If we cross strains with a similar tumor rate, the offspring inherits the tumor rate common to both parents; if both parents differ in tumor rate, the tumor rate of the offspring is on the whole intermediate between those of the parents. But all degrees of intermediacy are observed. In our material the number of strains in which the rate of the parent with the higher tumor incidence dominated was on the whole greater than the contrary one.

We selected for our hybridizations especially strains which differed markedly in their tumor rate and other characteristics and which had been followed over long periods of time and had been found consistent in their behavior. The English as a representative of a high tumor rate strain and the Cream as a representative of a low tumor rate strain were especially suitable for this purpose. In the majority of cases we selected few individuals for hybridization, either one male and one female or one male and several females. We followed the offspring through several generations. The near relatives of the

individuals used for hybridization were observed as to their tumor incidence and generally found to behave in a way characteristic of their strain.

Sometimes we hybridized sisters with the same male, or we used consecutively the same male with females from strains which differed much in their tumor rate. The results in the hybrids could usually be foreseen from the known tumor rate and tumor age of the parent up to a certain point of variability. The cases in which the strains used for hybridization had a similar tumor rate could be considered as controls. Here a similar tumor incidence ought to have appeared in the offspring and this is what usually occurred.

As we stated above, the results of hybridizations are typically intermediate, but the rates and tumor ages of the crosses may in some cases approach the parent with the higher rate, in other cases the parent with the lower rate.

We shall cite two examples, where the offspring resembled the parent with the higher tumor rate. (1) A son of a tumor mouse No. 240, belonging to the strain 8 + German, was mated to a White Cream female. 8 + German was a strain fairly rich in tumors and the particular family used had a tumor rate of 43 per cent. The tumors appeared early in life. In the White Cream used in this case tumors were extremely rare and they appeared late in life. Among the offspring 9 female mice lived long enough to be included in the records. Of these 9 mice, 5 died with tumors, 1 in the first and 4 in the second age period. In this case the influence of the father is undoubtedly very marked. In the Cream strain such a tumor rate was never observed even among isolated families. The tumor age of the hybrid is, however, in this case probably affected by the mother.

(2) In the second case which we wish to mention, an English Sable male belonging to the fourth generation of English Sable, who have normally a very high tumor rate, was mated to 3 females belonging to the substrain

Cream Y. In the substrain Cream Y the tumor rate had been zero. Four generations of the offspring were observed comprising altogether 68 female mice which reached an age sufficient for inclusion in our records. Thirty-six of these mice, that is, 53 per cent., died with tumors, 11 of these in the first age period. This is a record which comes near that of the English Sable. These as well as numerous other experiments seem to us more in harmony with the conclusion that multiple factors underlie the hereditary predisposition to mammary cancer in mice than the view of Maud Slye who maintains that the factor for mammary cancer in mice is a recessive monohybrid.

(4) The age at which tumors appear is just as characteristic of individual strains as the tumor rate. The tumor age is also transmitted by heredity. In some strains tumors appear relatively early, the percentage of tumors appearing in the first age period of life comprising the first twelve months is considerably greater than in others; and this characteristic is on the whole just as constant in the strains as the tumor rate as a whole.

We can distinguish two factors in the inheritance of the tumor age: (*a*) In general in the strains with the higher tumor rates the tumors appear at an earlier period of life than in the lower tumor rate strains. This comes out very clearly when we divide all the strains into three classes, those with a tumor incidence above 40 per cent., the high tumor rate strains; those with a tumor incidence between 20 per cent. and 40 per cent., the medium tumor rate strains, and those with a tumor incidence below 20 per cent., the low tumor rate strains.

If we determine in each class the percentage of tumors appearing in the different age periods, we find that the tumors appear the earlier in life the higher the tumor incidence and the difference between the different classes is quite marked.

There is in our case a definite relation between the factors, tumor age and tumor rate. We can interpret this

relation by assuming that a certain average quantity is inherited in the individuals of different strains which determines the intensity in the tendency towards the development of tumors. This intensity may depend on the average number and character of multiple factors favoring tumor growth which is characteristic of a strain. A special kind of factors or a larger number of factors causes both a higher incidence and an earlier appearance of cancer in a certain strain.

(*b*) In addition to this intensity which is a characteristic of the strains in general, there is a peculiar tumor age in certain strains which is independent of the tumor rate. Strains with a similar tumor rate may differ in their tumor age, and in hybrids tumor rate and tumor age may be inherited separately in the offspring. Thus two of our high tumor rate strains formed by the crossing of the same male (European 151) with two sisters (first and second daughter of No. 10, respectively) with tumor rates of 72 per cent. and 54.5 per cent., respectively, have relatively late tumors, in both only 15 per cent. of the tumors appearing in the first age period. In the Cream-English hybrids the tumor rates in two strains were similar and approximately intermediate, but in one of them the tumor age approached the late one of the cream parent; in the other it was nearer the early one of the Eglish parent.

We may therefore assume that in addition to the sum total of multiple factors which determine at the same time age and tumor rate, there are special factors which determine tumor age.

5. In general the cancer rate in mice is not a sex-linked character. Either the cancer rate and age of the father or mother strain may predominate in the cancer rate of the offspring. This fact does not, however, exclude the possibility that in certain cases a sex-linked factor may enter as one of the multiple factors which in all probability determine the inheritance of cancer. Certain of our observations suggest such a possibility. We found, for

instance, that in the Cream-English hybrids the mother strain was considerably more often predominant than the father strain; in addition we found that in reversing a cross different results were obtained in accordance with the difference in the tumor rate of the mother strain. It is, however, possible that these occurrences are chance phenomena and we offered this interpretation merely as a suggestion.

6. Our investigations make it possible to express in a quantitatively definite manner the hereditary tendency to cancer in individual strains of mice, the figures varying in different strains between zero and 100. This hereditary tendency is, however, not a simple quantity, but composite, because

(*a*) The hereditary disposition to cancer is probably due to the cooperation of multiple factors. The results of hybridization, which essentially were of an intermediate character, the fact that all kinds of intergrades between father and mother strain exist and that all possible variations in the hereditary tendency to cancer exist in different strains, and that the hereditary tendency determining the cancer age is not entirely identical with the tendency expressed in the cancer rate, very strongly suggest this conclusion. Variations in the number and character of the multiple factors in the different individuals may be responsible for the variations in intensity which determine the tendency to cancer in individuals, and different strains may differ as to the mean in the distribution of the factors among the individuals belonging to the strain. Thus we may assume that the strains English, $8\frac{1}{2} + 328$, European + I daughter of No. 10 have on the average a greater number of factors than the individuals of the strain Cream and German + 8 and many others; and it would be conceivable that in many cases a tumor mouse belonging to the strain English differs from a tumor mouse in the strain Cream, the former often exceeding the minimum of factors necessary for the production of tumors.

As far as the ordinary mammary cancer of the mouse is concerned, no definite proof has so far been brought forward to support the view that the hereditary tendency to cancer is due to the presence of a simple recessive factor.

(*b*) There is hidden in the figure expressing the tendency towards the development of cancer a second factor which is variable; namely, the activity of the ovary. In all the strains the realization of the hereditary tendency to cancer presupposes the activity of the internal secretion of the ovary. Without this cooperation no cancer can originate. With the full activity of this factor the hereditarily transmitted character for intensity of cancerous tendency determines the upper limit of the cancer rate. Again the intensity of this ovarian factor can be expressed in a quantitative manner, the quantity in this case representing the time during which the ovarian internal secretion had a chance to act. If through castration in the early stages of adult, sexually mature life, at the age of three to four months, this internal ovarian secretion is eliminated in mice, mammary cancer is practically prevented from appearing even in normally high tumor rate strains. The longer the ovarian function has a chance to act, the more the cancer rate increases up to the range which is given in the figure for the hereditary tendency to cancer. While we can thus experimentally lower the cancer rate of any strain, we do not so far know of a method which would permit us to raise the cancer rate above this point. The latter is almost reached if castration occurs at the age of eight to ten months. Suspension of breeding also diminishes somewhat the cancer rate in the great majority of the cases, but to a very much less extent than the exclusion of the internal secretion of the ovary, which latter is the true realizing factor, the cooperation of which is necessary. In one strain in which through segregation of the female mice breeding had been prevented the cancer rate was even higher in the non-breeding than in the breeding mice (9). Injury to the mamilla by the suckling young which Maud Slye believed

to be the external stimulus leading to the development of cancer in mice can therefore not be an important factor in the causation of mammary cancer in this species of animals. On the other hand, our demonstration of a certain influence of breeding on the cancer rate in mice adds another, though minor, factor to the internal secretion of the ovary, which latter represents, as we stated above, the principal realizing factor. Secondary realizing factors may therefore be added to this primary factor.

In principle, conditions are probably similar to what we determined in the case of the typical mammary cancer of the mouse in all other kinds of cancer. But we have to assume that the internal secretion of the ovary is substituted in other cases by other variable factors, which may be either internal secretions of a different kind or external stimulations. The latter play, as is well known, a very important rôle in the origin of cancer. They represent in addition a quantity which can be increased at will in contradistinction to the internal secretions and other inner factors. Thus through the use of external stimulation it may be possible to increase at will the cancer rate in certain kinds of cancers; in this way the hereditarily fixed intensity may become entirely obscured. Yet it can not be doubted that after all this factor is present even in these latter kinds of cancer, the best representative of which is perhaps the Roentgen ray cancer in man.

7. Thus it has become possible to express in a quantitative way the tendency to a disease, cancer. This tendency is due to the interaction of two main factors, both internal, the one hereditarily fixed and the other accessible to experimental variation. Both factors combined are more than the predisposition to cancer; they are in the case of this particular kind of cancer its essential cause. There may be, as we have seen, other factors superimposed upon these primary factors, like the effect of pregnancy; but they are not necessary, and the first two factors suffice for the development of mammary cancer in

the variable numbers which are characteristic of the different strains of mice.

8. Is it possible to associate the hereditary tendency to cancer with the other factors characteristic of particular individual mice or of strains of mice? We found in certain cases that from main strains substrains could be detached which differed from the main strain not only in color, but also in the tumor rate; the most noteworthy cases of this kind are the English Silver and Silver Fawn substrains, detached from the main English strain at an early period of inbreeding. In this case the tumor rates differed in a very pronounced manner from that of the main strain. But the connection between color and cancer rate or age is in this case, as in some other cases, an accidental linkage. There is no real causal connection between the color and the factors that determine cancer. It is apparently similar in the case of other characters such as vigor, prolificity, size and rapidity of growth. We find strains of all kinds among the high as well as the medium and low rate tumor mice. This comes out quite clearly in the case of the various English-Cream hybrids. Here the tumor rate and age may be quite similar, namely, intermediate in different crosses, and yet some of these strains may be vigorous, others frail; some prolific, others poor breeders. In crosses certain characteristics, such as wildness or tameness, vigor and resistance to disease, or frailty, prolificity or the opposite, are inherited, just as the cancer rate and the cancer age; but these characters may be distributed among the hybrids independently of the predisposition to cancer. However, it so happens that some of the most prominent low rate tumor strains are poor breeders, slowly growing, although vigorous mice, while some of the high rate tumor strains are prolific, more rapidly growing; but this relation does not seem to hold good in all cases and may therefore not be causal. Quite recently T. B. Robertson observed among his mice that especially the rapidly growing individuals were prone to become cancer-

ous and he believes that a causal connection between the developmental rate and tendency to cancer exists.

9. There may, however, possibly be an exception to this independence of the hereditary transmission of the tendency to cancer. As we have stated, we arranged our various strains of mice in three groups, in strains with a high, with a medium and low tumor incidence, and we found that in these three groups the cancer age varies *pari passu* with the decrease in cancer rate. If we now determine in these same three groups on the same percentage basis the age of death from all other causes taken together except cancer, we find the differences between the three groups considerably less than if we compare the percentages of the cancer age. There is, however, a distinct difference. In the group of the high cancer rate strains the age of death from all other causes but cancer is decidedly earlier than in the medium and low rate cancer strains. The difference between the medium and low cancer rate strains is very slight, very much less than that between the high and medium rate tumor strains, but this slight difference is again in the same direction. This relationship between cancer rate and age of death from other diseases may be explained in two ways: (*a*) We may assume that the mice dying from cancer are the strongest, most resistant individuals of the family or strain and those which are left back are therefore relatively less resistant to disease; the higher the cancer rate in a strain, the less resistant are the mice not dying from cancer, and the earlier, therefore, their age of death from other causes. Or (*b*) the majority of the strains in which the cancer rate was high happened to be less resistant strains and therefore the average age of death from other diseases is earlier. The average difference between the medium and low rate tumor strains, as far as general power of resistance is concerned, happened to be very small. Although it is perhaps impossible to decide definitely between these alternatives, we believe the second one to be much more

probable. If the first alternative were correct, we should expect to find a decided difference also between the age of death from other causes than cancer in the medium and low cancer rate strains. Here the difference is almost negligible. Furthermore, there are some strains with a very high tumor rate, but in which the rate of death from cancer in the first age period is relatively small. In those strains the resistant individuals would therefore be spared by cancer in the first age period; thus the resistant individuals would not be eliminated and the age of death from other causes should accordingly be late in these strains. Actually we find in such high tumor rate strains an early age of death from other causes. We may therefore conclude that in the material on which we base our conclusions the large majority of the high rate tumor strains were strains with a low general resistance to disease. While, as we have stated above, a high or low degree of resistance may be associated with either a high, a medium or a low cancer rate, this association of a low degree of resistance with a high cancer rate in a prepondering number of strains may possibly be more than a coincidence. Maud Slye states that cancer strains are the strongest strains, a conclusion at variance with our experience.

10. The tendency to die from other causes than cancer at a certain period of life, the resistance to disease in general, is also hereditarily transmitted, but as we have stated above it varies among different groups of strains much less than the predisposition to cancer. This should be expected if we assume that there exists besides a general power of resistance a special resistance or predisposition to individual diseases, and that the latter may vary among different strains and may thus to a certain extent balance each other in various strains.

Again the tendency to die from other diseases than cancer at a certain period of life depends upon the cooperation of the generative organs; but while in the disposition to mammary cancer the internal secretion of

the ovary is the main factor and suspension of breeding plays only a subordinate rôle, in the case of resistance to other death producing conditions, the suspension of breeding seems to be the main factor and the elimination of the ovarian function only a subsidiary factor which merely acts through the suspension of breeding which it calls forth or in which at least the suspension of breeding is by far the more significant factor. We found that the differences in the tendency to die from other causes than cancer which we observe normally between different strains of mice are entirely or at least to a great extent eliminated in mice which are prevented from breeding. All those strains in which breeding is prevented become long lived. If a difference in the duration of life should still exist between different non-breeding strains, it must be very much smaller than that between breeding strains. Furthermore, the difference in the longevity between non-breeding mice and castrated mice is likewise very small and this is the reason why we conclude that castration prolongs the life of mice mainly through its effect on breeding. As far as the cancer rate is concerned, on the other hand, we have shown that castration at an early age is much more effective than prevention of breeding.

11. In man it has been observed by several authors that in older individuals suffering from cancer a multiplicity of slight malformations, often due apparently to a misplacement of embryonal tissue, or a multiplicity of benign, or rarely even of malignant, tumors could be observed. Similar observations were made more recently by Goodpasture in the case of old dogs. It is usual to attribute these findings either to an inherited tendency to tumor formation in general in which imperfections in embryonal development play a certain part, and in which, as a result of this general tendency, various kinds of tumors develop in the same individual in its old age, or by some authors emphasis is laid on the importance of old age as such in the etiology of cancer; old age is sup-

posed to bring about a multiplicity of tumors or corresponding malformations.

In a similar manner Maud Slye states there is in mice inherited a general tendency to cancer. In hybrid strains, according to this author, this general tendency finds expression in the first hybrid generations in a tendency to develop sarcoma, while in subsequent generations more specialized tissues are affected which develop into carcinoma, and in still later generations multiple tumors are prone to appear.

We have not been able to observe such a cycle in our strains of mice. We had uniformly in all generations to deal with mammary carcinoma and in many autopsies which we made of tumor mice we failed to observe other kinds of tumors. This does not exclude the possibility or even probability that occasionally lesions may have been present in other mice which were tumors of a different kind. We described, for instance, a beginning squamous cell carcinoma in a mouse afflicted with a mammary cancer about 10 years ago; but on the whole such occurrences were rare and they could not be interpreted as due to the inheritance of a general tendency to cancer; in each case external factors would then at least partially determine which particular expression this general tendency should find.

In our strains there was inherited essentially, not a general tendency to tumor formation, but a specialized tendency to cancer of the breast. This does not exclude the possibility that in certain strains a tendency to the development of another kind of tumors may have been inherited side by side with the tendency to mammary cancer. In favor of this conclusion we may cite the experiences in cases of the so-called endemic occurrence of cancer, as, for instance, the cancer of the inner canthus of the eye in cattle observed by us in 1899, the cancer of the scrotum in the rat observed by Hanau, our observations of sarcoma of the thyroid gland in the rat. All these are instances of the inheritance of specific kinds of cancers.

The most striking confirmation of this view has in recent years been funished by Miss Slye, who discovered certain families of mice in which a tendency to special cancers, as, for instance, cancer of the liver, was inherited. We therefore conclude that inheritance to cancer consists in general in a tendency to the inheritance of a particular kind of cancer. This agrees also with the results of Miss Stark, who found in *Drosophila* two specific kinds of inheritable, tumor like formations originating by mutation.

12. Our continued investigations have thus borne out our earlier conclusion that the endemic occurrence of cancer among animals is due to this hereditary transmission of the disposition to cancer. In addition, infection with certain metazoon parasites which act as an external stimulus comparable to the action, for instance, of Roentgen rays, may play a part in certain cases; but even here the metazoon parasites seem to act on a basis of hereditarily transmitted disposition. The observations of Fibiger, with which the recent findings of Rohdenburg are in agreement, suggest this conclusion.

13. While these statements apply directly only to animals, the evidence on hand makes it probable that, in principle, conditions are similar in man; here also in all probability one or more factors are hereditarily transmitted which determine the intensity in the tendency towards cancer development. In man this tendency has, however, in many cases been more or less equalized among different families as a result of long continued cross breeding (10). Wherever this factor can be eliminated as among different races which remained relatively pure or among a very stationary population, as in certain parts of Norway, the evidence points to the conclusion that here too marked differences in the tendency towards cancer exist in various strains and races (11). Even among the ordinary population some occasional striking findings very strongly suggest this view.

Furthermore, Davenport (12) has shown that the tendency to neurofibromatosis is hereditarily transmitted as

a dominant. Similarly, the tendency to certain other tumors is undoubtedly inherited. The recent statistical studies of C. C. Little make it very probable that an inherited predisposition to cancer plays a part in human cancer in general (13).

As to the increase in the cancer rate which seems to be so general an occurrence, we may suggest that, so far as it is not due merely to improved diagnosis, it could be referred to a greater frequency in the dominance of the parent with a tendency to a higher tumor rate in the offspring.

As we stated above, such a condition of dominance was observed among our hybrid strains.

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DEVELOPMENT OF GONADS AND TRANSFORMATION OF SEX IN THE FROG

DR. EMIL WITSCHI

LECTURER IN ZOOLOGY IN THE UNIVERSITY OF BÂLE, SWITZERLAND

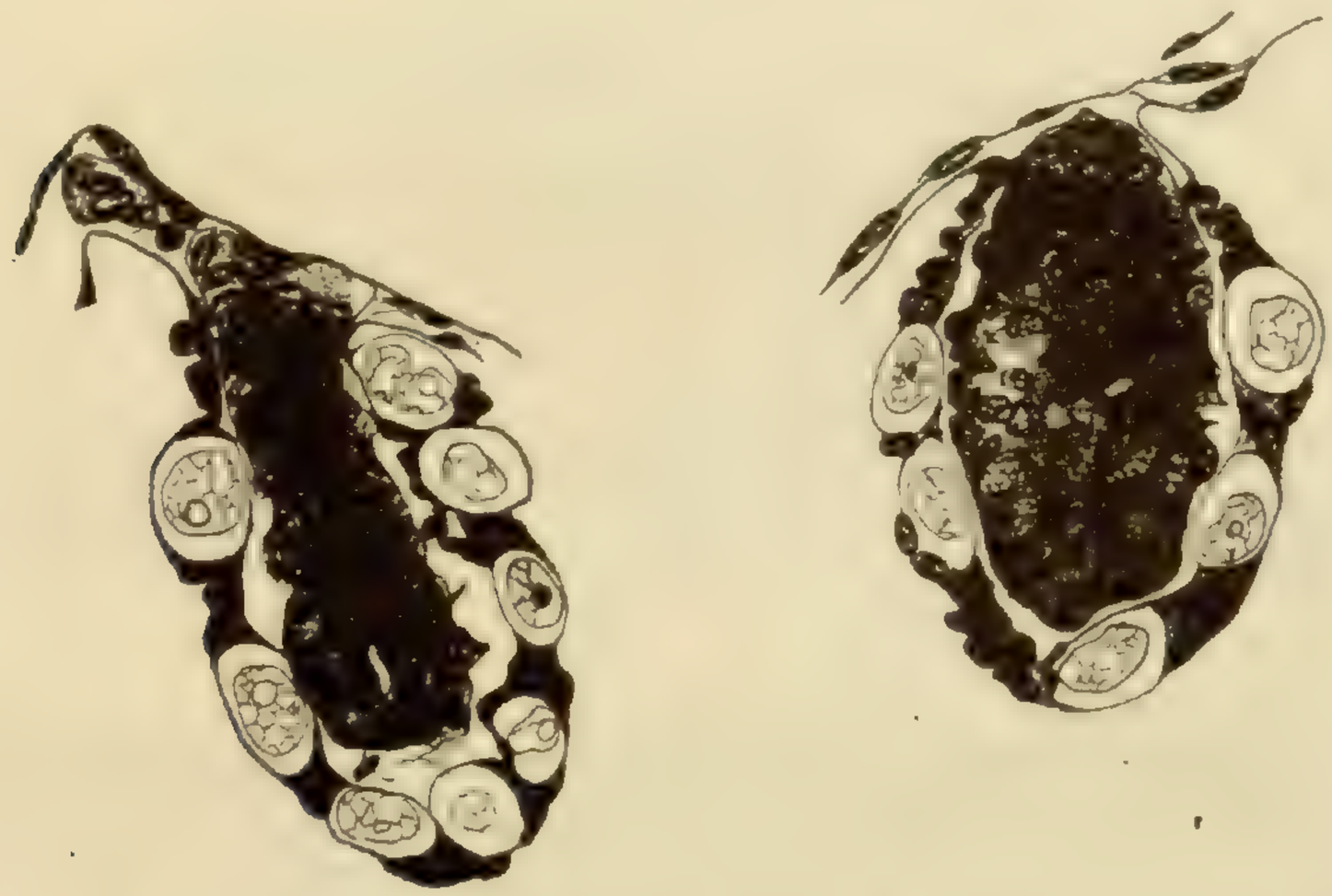
IN the following paper I intend to give a summary of the sexual problems in the frog, as they result from descriptive and analytic research. Richard Hertwig's successful experiments on sex-determination have directed the attention of investigators to this object. From 1911 to 1913 the writer was fortunate enough to have the opportunity of working in Hertwig's laboratory and since then the investigations have been continued at the zoological laboratory of Basle University. The present paper is a brief summary of the development of the sexual characters. In a later publication we hope to explain the experiments on sex-determination and to describe the chromosome cycle.

In the number of this journal for July–August, 1920, Dr. W. W. Swingle has published an interesting study on "Neoteny and the Sexual Problem," and in the February number of the *Journal of Experimental Zoology* the same author has described in greater detail the developmental history of the male gonad of *Rana catesbiana*. In both communications Swingle has disputed the correctness of the work of the Hertwig school. The following explanations may be regarded as a reply to Swingle's critical remarks.

A. THE SEXUAL GLANDS

1. *Morphology of the Undifferentiated Gland.*—The gonad of larvæ 22 mm. in length, just before sex-differentiation takes place, has the following structure. A simple germinal epithelium encloses the central primitive gonad cavity. Five to seven sex cords, budding from the

mesonephric blastema at regular distances, fill the cavity as shown in Figs. 1 and 2.



FIGS. 1, AND 2. Transverse sections through the two types of undifferentiated glands. Larvæ 22 mm. total length; 12 days old.

2. *The Ovary.*—The ovary is formed by direct development from the indifferent gonad. In consequence of a rapid increase of the germ cells by mitotic divisions the germinal epithelium thickens. In larvæ of 30–35 mm. total length the germ cells begin to be arranged in ovocysts. After a few simultaneous mitotic divisions the cells of the cysts enter the maturation stages. As Fig. 3 shows, the first cysts are found in the distal part of the gland. Towards the end of the larval period the formation of the oldest ovocysts is disintegrated. Each ovocytis having passed through the stages of pseudoreduction (synizesis, leptotene, pachytene) is surrounded by follicle cells and now enters the second period of growth. The nucleus increases considerably in volume and in the growing protoplasm numerous vitellogen granules appear (Fig. 7, sec. Oc.). Yolk is however not formed normally before the third or fourth season. The sex cords are of greater importance only in the male gonad, forming the testicular interstitium and the rete apparatus—while in the ovary each cord develops into an ovarial sac. As Fig. 1 shows, a little slit may appear already in the undifferentiated gland. More such are found when ovocysts

begin to be formed in the germinal epithelium (Fig. 3, *c*). But during the stage of metamorphosis the slits unite and form only one cavity: the secondary gonad cavity or ovarial sac. In consequence there are in each ovary 5 to 7 sacs, forming the thin gonad endothelium (Fig. 7, I, II).

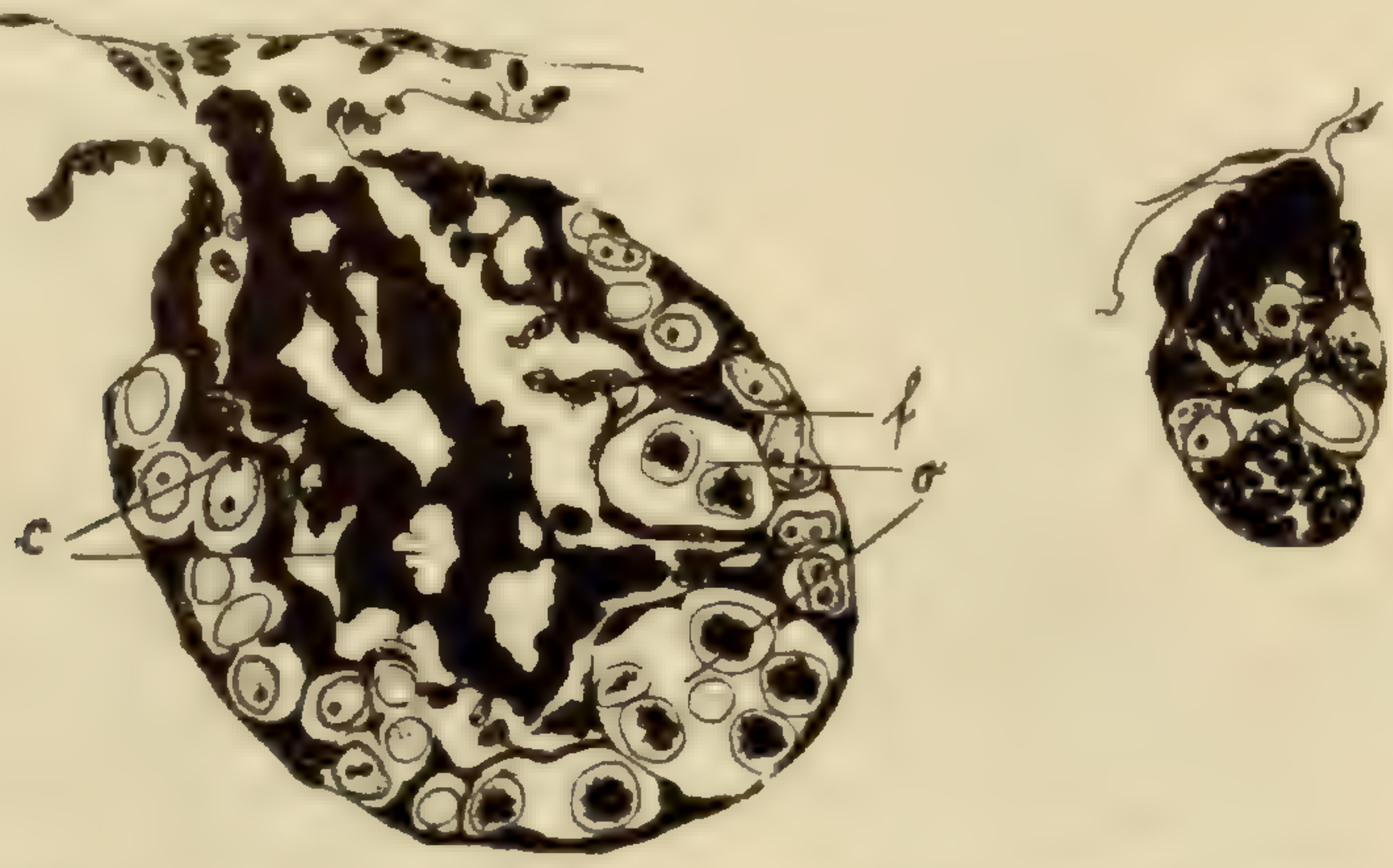


FIG. 3. Section through a young ovary. *C* = secondary gonad cavity. *O* = ovocysts. *F* = follicle cell. Tadpole 38 mm. total length.

FIG. 4. Transverse section through the earliest developmental stage of the testicle. Migration of the germ cells. *sp* = first germ cell in the sex cord (spermatogonia). Tadpole 22 mm. total length; 12 days old.

Characteristics: 1. *Persisting peripheral germinal epithelium.* 2. *First stages of ovocytes in the larval period.* 3. *Occurrence of a second growth period.* 4. *Presence of ovarial sacs.*

Therefore, precocious ripening of germ cells is not my "chief criterion of sex-differentiation," as Swingle says. On the contrary the other three are more characteristic, because they are completely absent in the male line!

3. *The Testis.*—In larvæ 22 mm. in length great differences in the size of the sex cords are found, as seen from a comparison of Figs. 1 and 2. Animals with the stout ones are undergoing transformation into males.

The testis is not formed by direct development from the indifferent gonad. *Its development begins with a change in the position of the germ layers.* The peripheral germinal epithelium having disintegrated, the germ cells cross the primitive gonad cavity and enter the sex cords (Figs. 4 and 5). The follicle cells migrate with the gonia

and only the simple peritoneal epithelium remains (Fig. 5).

Shortly after this migration the canaliculi seminalis are formed, at first as irregular slits, but already during metamorphosis they develop into characteristic radiating tubes. At all times they remain connected with the sex cords. These on their part give rise to the rete vasculosum Halleri and the connections with the mesonephros (Vasa efferentia testis).[1]

In the following years the testes grow very slowly until the fourth season, when a rapid increase due to active mitotic divisions of the spermatogonia begins. The spermatic tubes become convoluted; spermatocysts are formed and in the month of July the maturation cycle begins. In August and September the spermatozoa are developed.

In only one case have I found an abortive præspermatogenesis in a three-year-old grass frog (*R. temporaria*). But as Champy has observed, similar degenerating maturation cycles are frequently formed in water frogs (*R. esculenta*) in the second year. According to Swingle precocious ripening of male germ cells in the bull frog (*R. catesbeiana*) already occurs in first year larvæ, and ripe spermatozoa are formed in second year animals.

Characteristics: 1. *Central germinal layer.* 2. *First ripening stages giving rise to functional spermatozoa in the fourth season.* 3. *The maturation divisions directly succeed the pseudoreductional stages.* (There is no second growth period.) 4. *Rete apparatus and Vasa efferentia testis.*

4. *Hermaphrodism.*—It is a strange and interesting fact that the typical development of the testis as just described is rarely observed. Under natural conditions most individuals develop first ovaries which later on are transformed into testes. During this transformation the gonads, showing the characteristics of both sexes, are hermaphroditic; but without exception the female

[1] Cf. Witschi, 1914.

characters become reduced and mostly disappear completely. In Fig. 6 we have a transverse section through a larval hermaphroditic gland. As in the young ovary (Fig. 3) the distal part contains ovocysts with ovocytes in the synizesis stage, and in the sex cord is found the second gonad cavity or ovarial sac. But the transforma-

FIG. 5. Cross-section through a young male gonad. Leaved germinal epithelium. All germ cells in the sex cord.

FIG. 6. Transverse section through a larval hermaphroditic gland. Female germinal epithelium; ovarial sac. At (*i*) migrating germ cells.

tion has already begun. The sex cord is more compact than usual and its middle part is penetrated by immigrating germ cells from the basal end of the germinal epithelium. These germ cells after their entrance into the sex cords are to be called spermatogonia.

After the metamorphosis the whole female germinal epithelium undergoes degeneration (only the peritoneum is preserved) while the central testis anlage develops into a normal male gonad.

Sometimes great irregularities are observed. It occurs occasionally that one gonad undergoes the transformation of sex earlier than the other in consequence of which such animals pass through a stage of lateral hermaphrodism. Such cases have often been described and are, I believe, of the greatest interest with regard to the development of somatic sex characters. But even within the same gonad differences can be found. Sometimes the

transformation begins at one pole and proceeds continuously to the other. Fig. 7 shows a longitudinal section of such a hermaphroditic gonad. The small testicular part at the posterior end could easily be distinguished macroscopically. The fifth sex cord is almost a normal male one. Numerous spermatogonia are scattered throughout. The slits, which are seen, represent the beginnings of the spermatic tubes. At (*a*) a bridge of tissue

FIG. 7. Longitudinal section through a hermaphroditic gonad. Transformation of sex proceeding from the posterior to the anterior pole. *I–V* = the five sex cords. *sec. oc* = ovocytes in the second growth period.

is seen, connecting the cord with what is left of the female germinal epithelium. In the fourth cord the transformation has not progressed as far as in the fifth. The center

is occupied by the shrinking ovarial sac with a degenerating egg. The spermatogonia are not numerous but they are already dispersed throughout the wall of the ovarial sac. The germinal epithelium contains a degenerating egg and an ovocyst. The third cord shows the beginning of the process of transformation. It is greatly enlarged, but only a few spermatogonia have migrated into its basal part. Numerous degenerating eggs are scattered over the distal tissue of the sex cord. The germinal epithelium is disintegrating. The second and the first sex cords show the typical proportions of an ovary: wide and thin-walled ovarial sacs and well-developed germinal epithelium. The ovogonia adhere to the peritoneum, the larger eggs projecting inwards. Thus the walls of the sacs are folded on the outside. This preparation is taken from a first year frog, several months after metomorphosis. The transformation of sex in the grass frog often occurs in the second year but likewise is sometimes found in adult animals. Eggs scattered through the testicular tissue have been frequently observed, but ignorance of the development of the gonad has produced the belief that they were derived from spermatogonies. Recently Levy and Swingle claim that these "so-called" eggs only are enlarged ovocyte-like male germ cells; and Swingle believes that "the so-called sexually indifferent or sexually intermediate forms of the Pflüger-Hertwig school are very probably male animals whose germ cells show precocious ripening as far as the pachytene stage" (1920); and "as a consequence of this curtailment of the maturation cycle to the early stages of the process, without exception these writers (Witschi and others), being unable to differentiate male from female, concluded that all frog tadpoles first develop as females, then later half of the female tadpoles must transform into males. . . ." (1921).

It is evident that Swingle has misunderstood our previous communications. In *Rana temporaria* the ovocytes do not always degenerate after the pachytene stages but

enter the second growth period (Fig. 7). It is difficult to understand when Swingle says that R. Hertwig, Kuschakewitsch and Witschi "concluded that all frog tadpoles first develop as females." On the contrary I have (1914) described the typical (or direct) development of the testicle as it is to be observed in the alpine *Rana temporaria*. Under optimal conditions of temperature in this variety of the grass frog already after the twelfth day (larvæ 20 to 22 mm. total length) 50 per cent. males are found; in such cultures transformation of sex never occurs.

In his material Swingle has not seen this typical development of the testicle. The described first and second year males are in reality hermaphrodites. His photograph 33, plate 4 (1921) does not show a transverse section through a male but through the female gonad of a first year tadpole, characterized by the ovarial sac (secondary genital cavity) and the peripheral germinal epithelium. Photograph 34 likewise is not taken from a male gonad but from a hermaphroditic one. It shows the same stage of transformation of an ovary into a testicle as Fig. 45 in our publication (1914): representing the gross structure of an ovary, but in the thickened wall of the ovarial sac are several immigrated germ cells (spermatogonia). Photograph 35 illustrates another type of transformation, characterized by an excessive proliferation of the sex cords, as is likewise described in our publication (1914, Fig. 41).

The cytological facts described in great detail by Swingle will be discussed in another communication. They do not alter our view of the significance of the developmental changes.

If there should still remain any doubt in regard to the correctness of my interpretation the following account may help to dispel it.

B. The Somatic Sex Characters

1. *The Müllerian Duct.*—The oviduct first appears as a dense cell cord close to the lateral margin of the kidney. Already in the first sumner the oviducts from the ostium abdominale tubæ to the opening in the cloaca are completely formed. They are thin walled, contain a small lumen, and run straight backwards. In the second year they grow slowly and move away from the ureters. Ordinarily in the third year a large longitudinal growth begins and the duct now becomes folded, as is well known from the anatomy of adult frogs.

In males which show a typical development of the testicles, no Müllerian ducts of any significance are formed. On the other hand, such animals as first develop ovaries and later undergo the transformation of sex, also show regular oviducts; and these continue to grow just up to the time when the transformation of sex begins. *This parallelism in the behavior of the Müllerian ducts and the gonads furnishes definite proof that the "eggs" and "ovocytes," described by the writer, are in fact really eggs and ovocytes and that the transformation of sex is a well-established fact.*

After the transformation of sex, when the ovocytes have disappeared, the Müllerian ducts begin to shrink, but they do not disappear completely, and such reduced oviducts of various sizes are often found in adult male frogs.

Regarding the question of the relations between somatic characters and the gonads, the lateral hermaphrodites furnish most interesting information. The Müllerian ducts are always developed in correlation with the gonad of the same side. Lateral hermaphrodites show always well-developed oviducts on the ovarial side and smaller ones on the testicular side. The correlation is therefore independent of the action of hormones.

2. *The Vesicula Seminalis and the Thumb Cushion.*—The male somatic characters appear chiefly in the second year and always develop symmetrically, in lateral her-

maphrodites, both sides depending from the first formed testicle. They seem, however, not to be influenced by internal secretions, as all experiments gave negative results. We will not enter here into this much discussed problem, but refer the reader to our publication on hermaphrodism.

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THE RATE OF GROWTH FOLLOWING AN INITIAL PERIOD OF SUPPRESSION[1]

DR. HOWARD S. REED

DIVISION OF PLANT PHYSIOLOGY OF THE UNIVERSITY OF CALIFORNIA
CITRUS EXPERIMENT STATION

THE present paper attempts to discuss quantitative aspects of the growth of animals which, though eventually reaching the same approximate size, reached that size at widely different ages. The data upon which the discussion rests have been drawn from the published articles of several workers in the field of animal physiology and deal with the growth, under varying conditions, of the albino rat.

When the growth of an animal is suppressed for a long time the capacity to grow persists, even beyond the period at which growth ordinarily ceases in that species. The studies of Osborne and Mendel have amply demonstrated the existence of this capacity to grow and to reach the weight characteristic of mature individuals of their species. The growth impulse is something inherent in the organism. The environment, while modifying the amount of growth, has less influence upon the specific character of the growth of organisms than has the essential constitution of the living substance. A quantitative study of the growth rate of organisms ought, therefore, to lead to considerations of a fundamental nature.

The nature of the growth rate in general is revealed by the use of a few simple equations of the first order. They show that growth proceeds at a rate similar to that of a monomolecular reaction. Robertson[2] and others have discussed growth in its relation to autocatalysis. In a recent paper I have compared the equations of slowly and rap-

[1] Paper No. 84 from the University of California Graduate School of Tropical Agriculture and Citrus Experiment Station.

[2] Robertson, T. B., *Arch. Entwicklungsmech. d. Org.*, **37**: 497–508. 1913.

idly growing apricot shoots. In each case the rate was proportional to a function of the final length of the shoot. The shoots which had a greater final length at the end of the season grew more rapidly from the start than their shorter neighbors, though the growing periods of the two samples were the same. The particular point of interest lay in the fact that the equations representing the growth rates had the same value for the constant of the reaction, differing only in the value of the constant expressing the final length of the shoots.

The present paper attempts to supplement this work by investigating the growth of organisms which reached approximately mature size after being subjected to conditions which suppressed growth in early life.

In the former case the difference between the two lots was in their final size; in the present case the difference between the two lots was in the time required to make equivalent body weight.

A. The Rate of Growth of Rats on Adequate Diets

The growth of the white rat has been so completely studied by many investigators that no extended discussion of the subject is required.

The rate of growth of rats varies slightly in different lots, but in general it follows the course of a differential equation. In later paragraphs I shall show that the equations used are those which express an autocatalytic reaction. The rate at which each sex grows is quite characteristic. The females grow relatively faster in early life than the males, come sooner to maturity, and weigh less at maturity than the males.

The growth of a white rat in the first year comprises two cycles. The first cycle, covering approximately 150 days, consists of a rapid increase in the weight and size of the body. The second cycle, covering the remaining 200 days, consists of a thickening of the body and a deposition of fat. The growth of rats in each of these cycles may be expressed by the equation

$$\log \frac{x}{a-x} = K(t-t_1),$$

in which x represents the weight of the animals at time t, a represents their weight at the end of the cycle, t_1 is the time at which the weight, x, is one half a, and K is a constant.

Although the quantitative relationships of this growth rate have been ably discussed by Hatai,[3] it will be shown subsequently that there are numerous reasons for using the above-mentioned formulas for computing growth. The computation of these and other growth rates studied in this paper have been made with the aid of tables published by Robertson.[4]

Table I contains data on the growth of white rats in the first year of life. The data for the rats were taken from Donaldson's tables 63 and 64.

The equations for the growth of the animals are as follows:

Males, first cycle, $\log \frac{x}{228-x} = .0187\ (t-73)$

Males, second cycle, $\log \frac{x-220}{280-x} = .0123\ (t-213)$

Females, first cycle, $\log \frac{x}{170-x} = .0211\ (t-61)$

Females, second cycle, $\log \frac{x-170}{226-x} = .0086\ (t-191)$.

It will be noticed that the calculation of the second cycle involves a change of the axes of the coordinates so that the new point of origin is near the point at which the first cycle of growth ended. The first cycle of growth in the female appears to terminate somewhat earlier than that in the male and the value of K, the constant, was greater in the growth curve of the female. These relations agree with the repeated observation that in early life the female grows more rapidly than the male. In the second cycle the female grows less rapidly than the male.

The close agreement between the observed and the cal-

[3] In Donaldson, *loc. cit.*

[4] Robertson, T. B., Univ. Calif. Publ. Physiol., 4: 211–228. 1915.

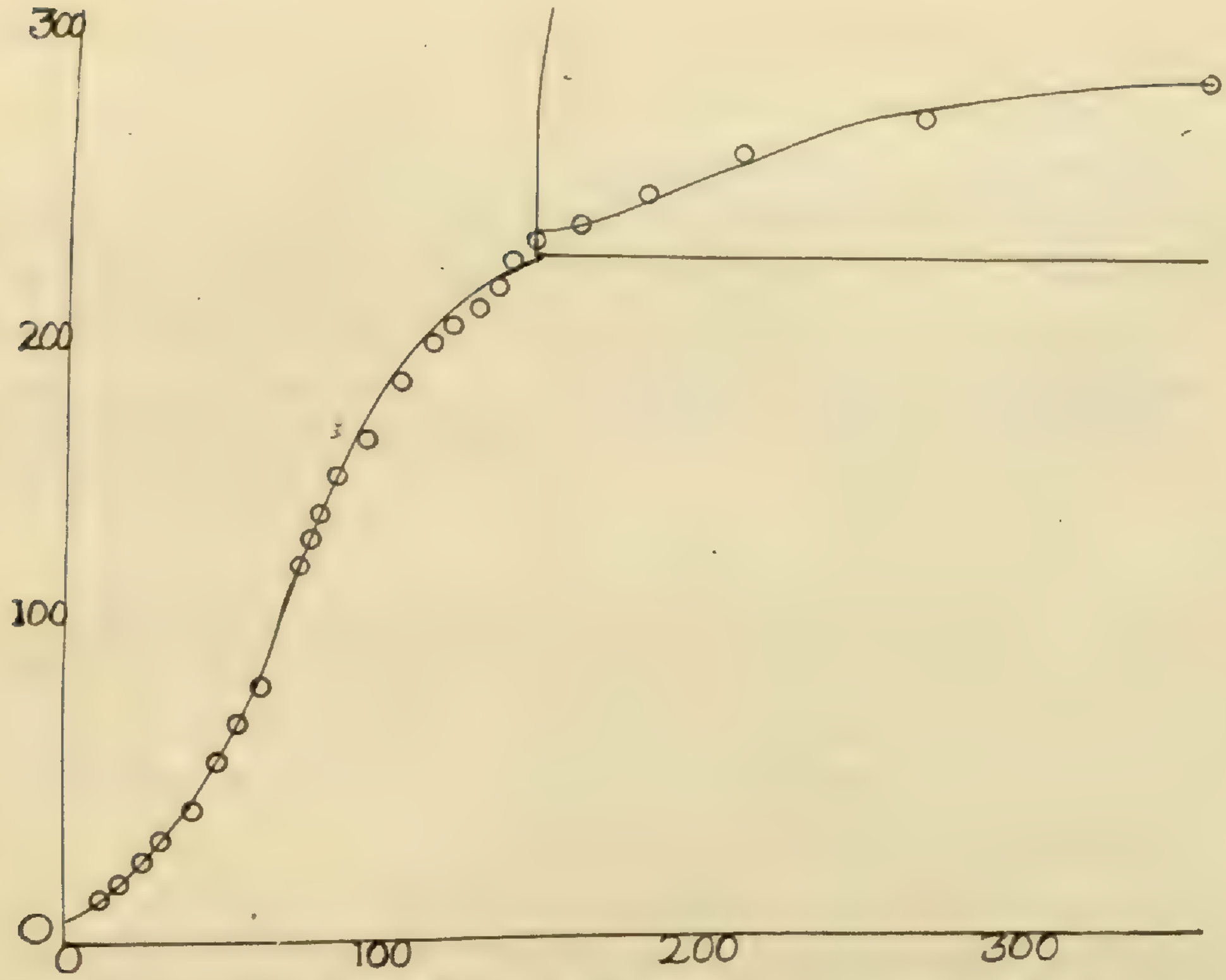

FIG. 1. Curve of growth for male white rat. . . . . observed weight; ——, calculated weight. The weight for days 0–150 was calculated from log $[x/(228-x)] = .0187\ (t-73)$. The weight for days 150–365 was calculated from log $[(x-220)/(280-x)] = .0123\ (t-213)$.

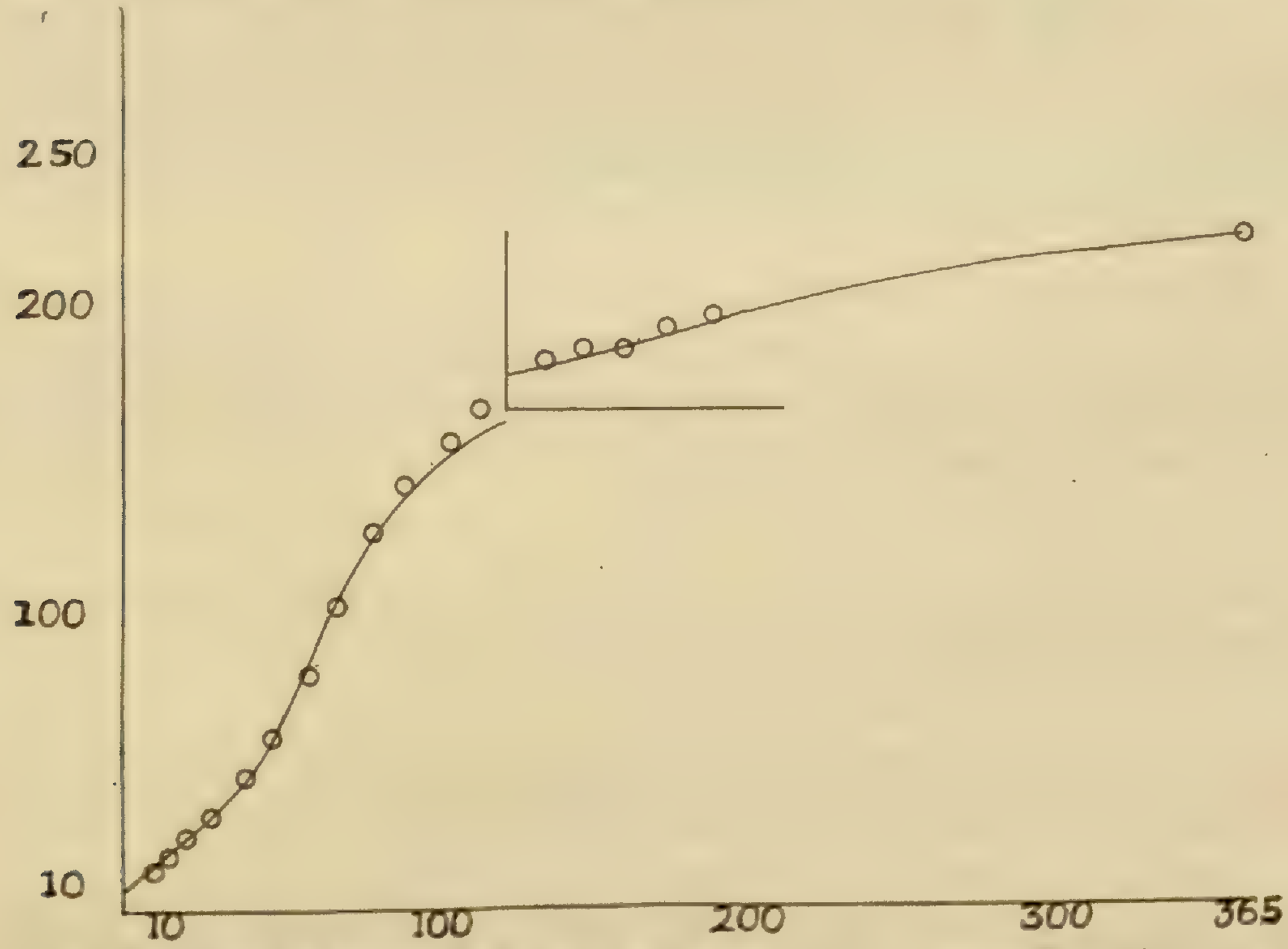

FIG. 2. Curve of growth for female white rat. . . . , observed weight; ——, calculated weight. The weight for days 0–124 was calculated from log $[x/(170-x)] = .0211\ (t-61)$. The weight for days 124–365 was calculated from log $[(x-166)/(226-x)] = .0086\ (t-191)$.

culated weights of the animals is shown by Table I and by Figs. 1 and 2.

B. The Growth of Rats Recovering from an Initial Period of Suppression

The published work of Osborne and Mendel contains very convincing evidence that the white rat possesses an inherent capacity for growth and that this capacity to grow survives long periods of suppression due to inadequate nutrition. Rats whose growth had been suppressed for over a year made prompt response and quickly reached mature size when the inhibiting factors were removed.

TABLE I

Growth of Albino Rats during their First Year [5]

Males Weight			Females (Unmated) Weight		
Age (Days)	Observed (Grams)	Calculated (Grams)	Age (Days)	Observed (Grams)	Calculated (Grams)
First cycle			First cycle		
11	13	15	11	13	14
15	17	17	15	18	16
21	21	22	21	23	21
31	32	32	29	31	30
40	42	44	40	44	45
49	57	60	49	58	61
61	82	85	61	78	85
70	107	107	70	100	103
79	128	129	82	125	125
85	144	143	92	140	139
97	160	168	107	155	154
107	177	185	117	167	159
117	191	198	124	171	162
131	203	211	Second cycle		
143	218	217	124	171	179
150	225	220	131	179	180
Second cycle			138	182	182
150	225	229	143	183	183
157	227	230	150	185	184
164	231	232	164	185	188
171	236	234	178	192	192
178	239	236	192	196	196
185	240	239	365	226	224
216	253	251			
256	265	266			
365	279	279			

[5] Data from tables 3 and 64 in: Donaldson, H. H. "The Rat." Philadelphia, 1915.

A second feature of their results, which is no less noteworthy than the first, is the rapidity of growth after adequate diets were given. They show that the gains made by the rats whose growth had been previously suppressed were made in much less time than would be required for a rat on adequate diet to make the same gain in weight.[6] This inquiry is concerned only with certain characteristics of the rate of growth following the initial period of suppression and will not attempt any discussion of the nutritional aspects of the problem. The data discussed have been drawn from the work of Osborne and Mendel. It has not been possible to obtain records of enough individuals to give a statistically reliable average, yet the records employed are fortunately free from extreme fluctuations and are satisfactory as far as individual records can go.[7]

The first case to be discussed is that of a male rat (No. 1012) which at age 370 days had reached a body weight of 127 grams, having been fed alternately "gelatin food" and "milk food."[8] On the 368th day the ration was permanently changed to "milk food plus mixed food." This change in diet was promptly followed by rapid growth and the attainment of mature weight about 180 days later. It is evident from Osborne and Mendel's chart that the curve of "resumed growth" was steeper than the normal curve of growth. This difference is especially well marked during that portion of the time in which there is an actual increase in body size and less well marked during the time in which its increase is due to the formation and deposition of fat.

A quantitative study of the resumed growth of this animal shows the existence of two distinct cycles, each of which is expressed by an equation of the type already

[6] Osborne, T. B., and Mendel, L. B., *Amer. Jour. Physiol.*, **40**: 16–20, 1916.

[7] The writer is greatly indebted to Dr. Osborne and Professor Mendel not only for their kindness in furnishing data, but for their criticism of this manuscript.

[8] Osborne, T. B., and Mendel, L. B., *Jour. Biol. Chem.*, **18**: 95–107, 1914, Chart V.

discussed. From the time at which an adequate diet was supplied, until about the 432d day, the growth is expressed by the equation

$$\log \frac{x}{220 - x} = .0193\ (t - 363).$$

This indicates a cycle of growth which was completed at a body weight of 220 g. and which overlapped slightly

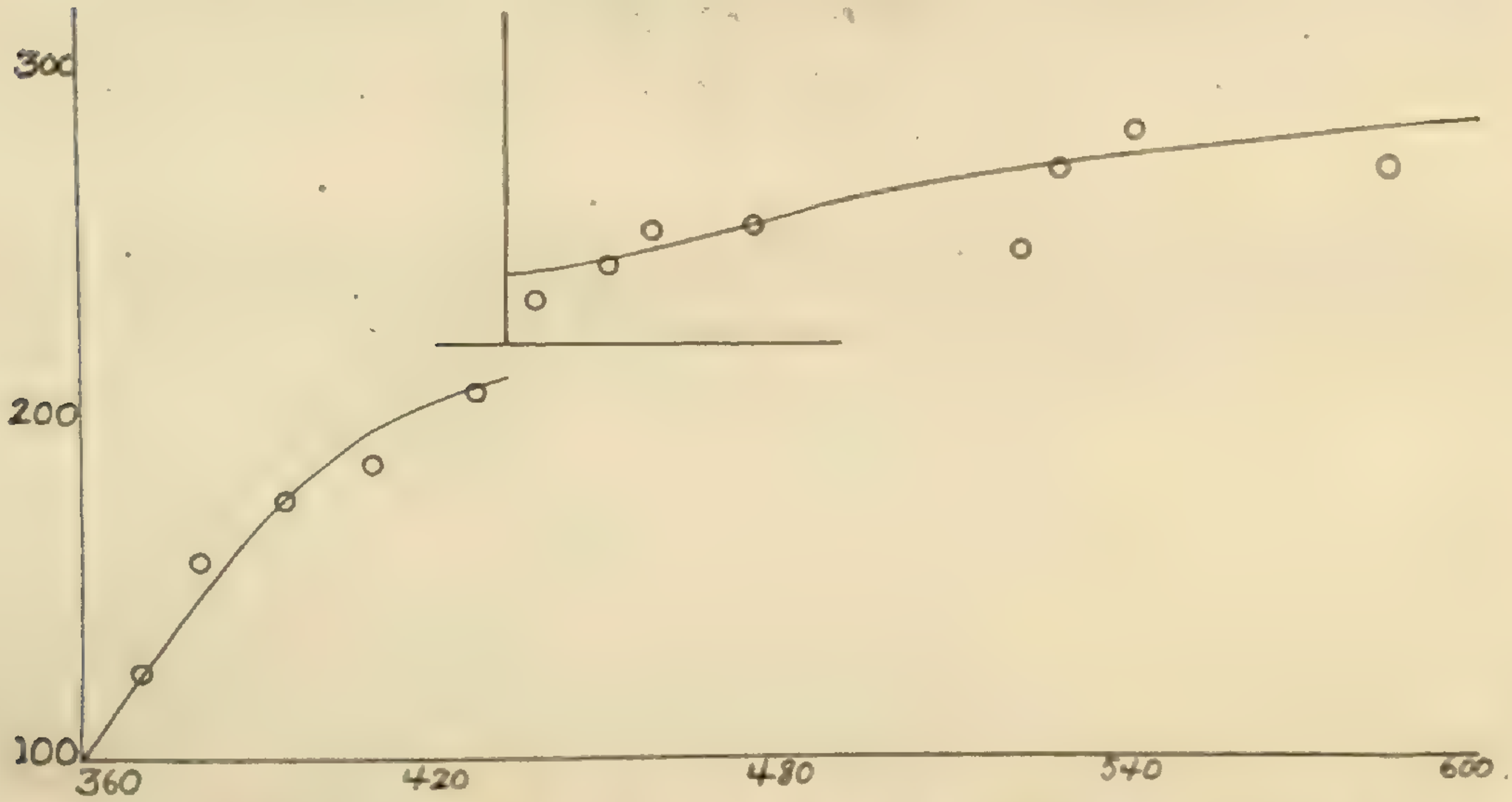

FIG. 3. Curve of growth of a male white rat following a suppression of 370 days. . . . . , observed weight; ——, calculated weight. The weight for days 360–432 was calculated from log $[x/(220-x)] = .0193\ (t-363)$. The weight for days 433–600 was calculated from log $[(x-220)/(288-x)] = .0091\ (t-475)$.

the second cycle of growth which was completed when a body weight of 288 g. had been attained. The equation representing the second cycle of growth is

$$\log \frac{x - 220}{288 - x} = .0091\ (t - 475).$$

The second cycle may be represented by a curve having ordinate and abscissa axes originating at $y = 220$ and $x = 432$. The values are shown in Fig. 3. The agreement between calculated and observed values is as good as could be expected with only one animal in the sample.

The next case to be noted is that of a male rat (No. 2161) which was stunted by a diet of inadequate protein from age 38 days to age 248 days.[9] During this

[9] Osborne, T. B., and Mendel, L. B., *Jour. Biol. Chem.*, 23: 439–454, 1915.

period the increase in body weight was from 53 to 73 g. 220 days after an adequate diet had been begun the rat had grown to a weight of 300 g. The first cycle came to an end at the 368th day, when a weight of 230 g. had been reached, and is expressed by the equation

$$\log \frac{x}{236 - x} = .0180\ (t - 285).$$

The second cycle of growth is represented by the equation

$$\log \frac{x - 225}{305 - x} = .0150\ (t - 408).$$

For a graphic representation of these values see Fig. 4.

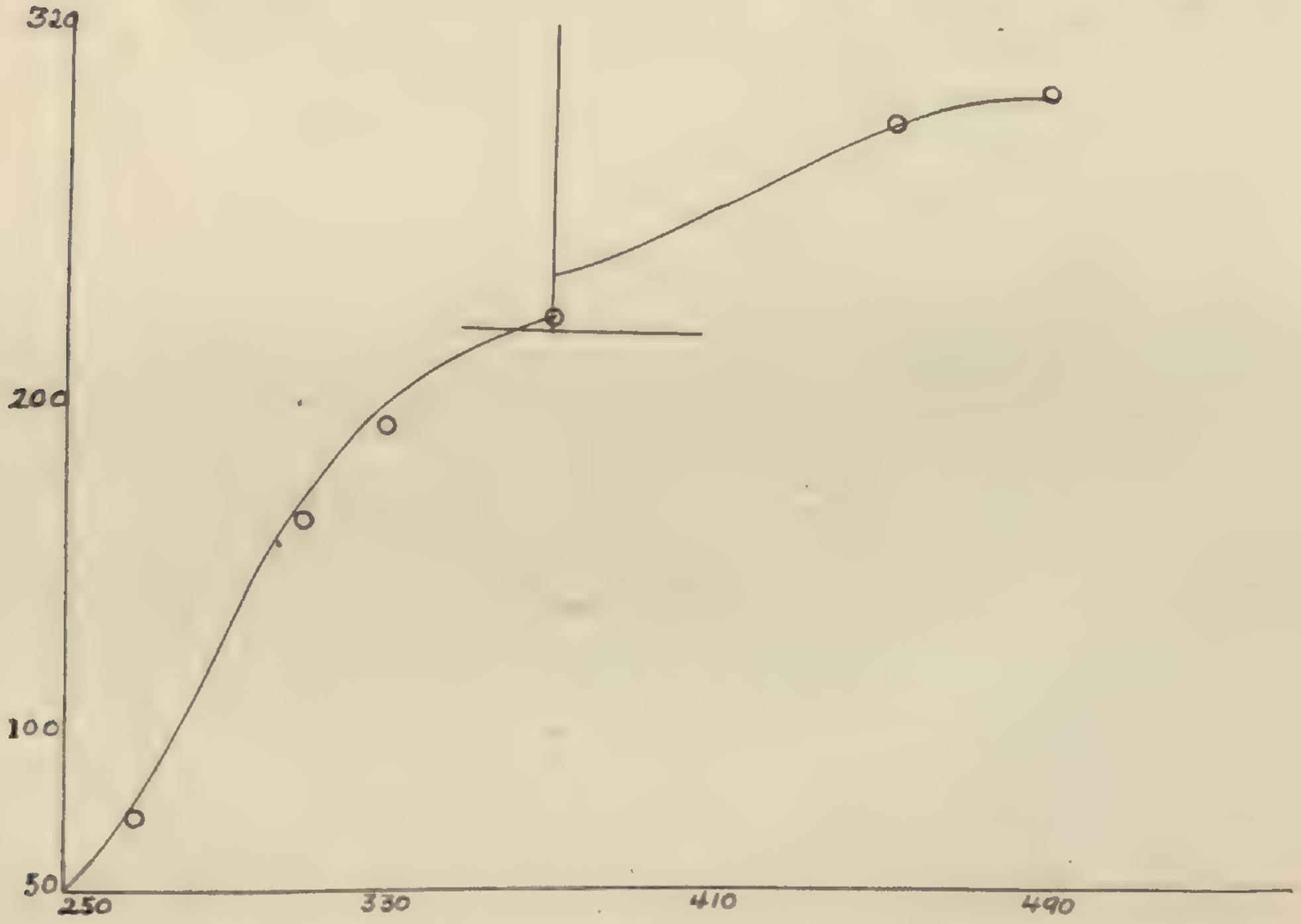

FIG. 4. Curve of growth of a male white rat following a suppression of 248 days. . . . . , observed weight; — — , calculated weight. The weight for days 248–368 was calculated from $\log [x/(236 - x)] = .0180\ (t - 285)$. The weight for days 368–488 was calculated from $\log [(x - 225)/(305 - x)] = .0150\ (t - 408)$.

The third case studied was that of a female white rat (No. 2033) whose growth had been retarded by limiting the daily quantity of food.[10] Although the weight of this animal was held below 60 g. for the remarkably long period of 510 days, it had not lost the capacity for growth.

[10] Osborne, T. B., and Mendel, L. B., *Jour. Biol. Chem.*, **23**: 439–454, 1915. Also *Amer. Jour. Physiol.*, **40**: 16–20, 1916.

After 135 days on an adequate diet it weighed 222 g.

The equations for the first and second cycles of growth of this animal are

$$\log \frac{x}{175 - x} = .0260\ (t - 519)$$

and

$$\log \frac{x - 170}{230 - x} = .0231\ (t - 610).$$

For a graphic representation of these values see Fig. 5.

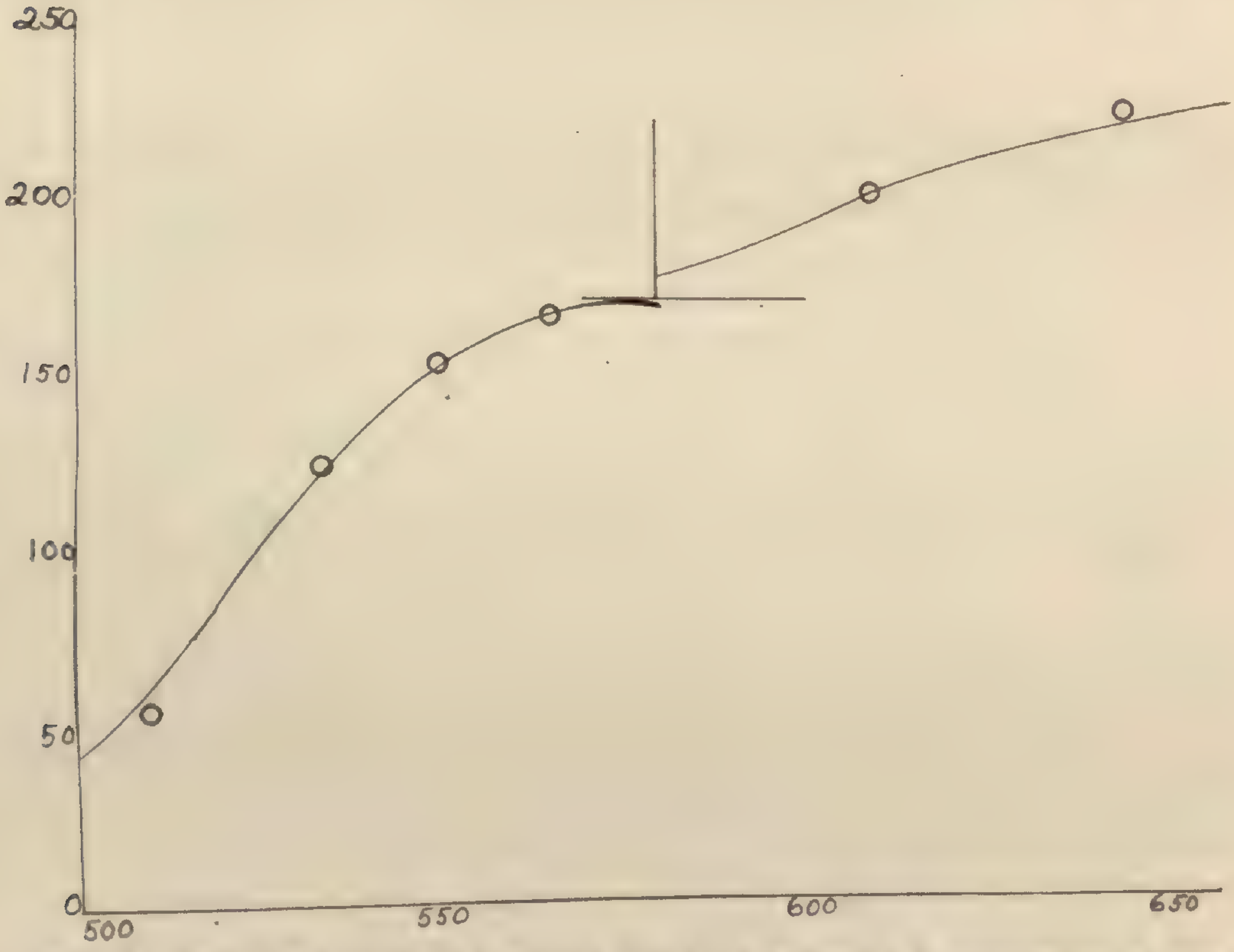

FIG. 5. Curve of growth of a female white rat following a suppression of 500 days. . . . . , observed weight; ——, calculated weight. The weight for days 500–580 was calculated from log $[x/(175-x)] = .0260\ (t-519)$. The weight for days 580–660 was calculated from log $[(x-170)/(230-x)] = .0231\ (t-610)$.

The constants of the different equations are grouped for convenience in Table II. From them it appears that male rats fed on adequate diet reached a weight of 280 g. at the end of their first year, and the female rats a weight of 230 g. in the same time. The females grow relatively faster in the first cycle than the males and in harmony with this property the values of K (the constant of the reaction) in the first cycles were greater than

those of the males. The values of K in the second cycles were less than those in the first cycles. In the case of males on adequate diet the value of K in the second cycle is about 66 per cent. of its value in the first, but in the females the value in the second cycle is only 33 per cent. of that in the first cycle.

The values of the constants are somewhat different in the cases of rats subjected to an initial period of suppression. Rat No. 1012 reached approximately the same body weight as a male rat fed continuously on adequate diet. Its weight was 127 g. when full feeding began on the 368th day; the gain was, therefore, 153 g. in 212 days. The male rats on adequate diet weighed 127 g. at the 77th day; their gain of 153 g. was, therefore, made in 286 days. The animal recovering from initial suppression, therefore, required considerably less time to make an increase from 127 g. to 280 g., than adequately fed animals required to reach the same stage of development.

The case of No. 2033 (female) has special interest because of the remarkably long period of stunting. The period of suppression started on the 39th day when the rat weighed 53 g. and ended on the 510th day when it weighed 57 g. The animal attained a weight of 222 g. in 135 days, following the resumption of full feeding. A female rat on adequate diet from the time of weaning would require 295 days to increase from 57 g. to 222 g., or more than twice the time to make the same gain. Other records of the time required by stunted and by adequately fed animals to attain a given weight are given by Osborne and Mendel[11] and in all cases the time was greatly reduced in the case of animals recovering from stunting. These authors likewise pointed out the broad biological significance of this faster growth rate. This question of rate is one of extreme interest in connection with the dynamical aspects of growth

[11] Osborne, T. B., and Mendel, L. B., *Amer. Jour. Physiol.*, 40: 16–20, 1916.

TABLE II

COMPARISON OF CONSTANTS

	First Cycle		Second Cycle		Duration of both Cycles
	a	*K*	*a*	*K*	
On adequate diet. Male	228	.0187	280	.0123	365 days
On adequate diet. Female	175	.0234	230	.0076	365 days
On restricted diets:					
No. 1012. Male	220	.0193	288	.0091	263 days
No. 2161. Male	236	.0180	300	.0150	286 days
No. 2033. Female	175	.0260	230	.0231	162 days

C. RATES OF GROWTH AS COMPUTED FROM VALUES OF dx/dt

It is unnecessary to dwell upon the prime importance of the study of rates in physiological investigations. We are concerned not only with what the organism is, but how it came to be what it is. As soon as we begin to study the problem of development, we encounter the question of rates. No better means of studying the rate of change in a system has yet been found than the differential calculus.

The differential equation representing the rate of autocatalysis is

$$\frac{dx}{dt} = kx\,(a - x).$$

a, x and t represent the same values as before, but $k = K/a$. We may proceed, therefore, to examine the derivatives of the equations used above to express the sizes of the animals at various time intervals. The values obtained for the growth of males and females are shown in Figs. 6 and 7 in comparison with the observed weekly increases of the animals which have been studied. The computed values were obtained from the tables published by Robertson[12] which give the values of $(1/Ka) \cdot (dx/dt)$ for corresponding values of $K(t - t_1)$.

The rate of growth of male rats on adequate diet was computed for each cycle from the figures in Table I. The

[12] Robertson, T. B., Univ. Calif. Publ. Physiol., 4 : 211–228, 1915.

rates for each cycle are shown in Fig. 6. The growth rate at birth had an appreciable value, as one might expect.

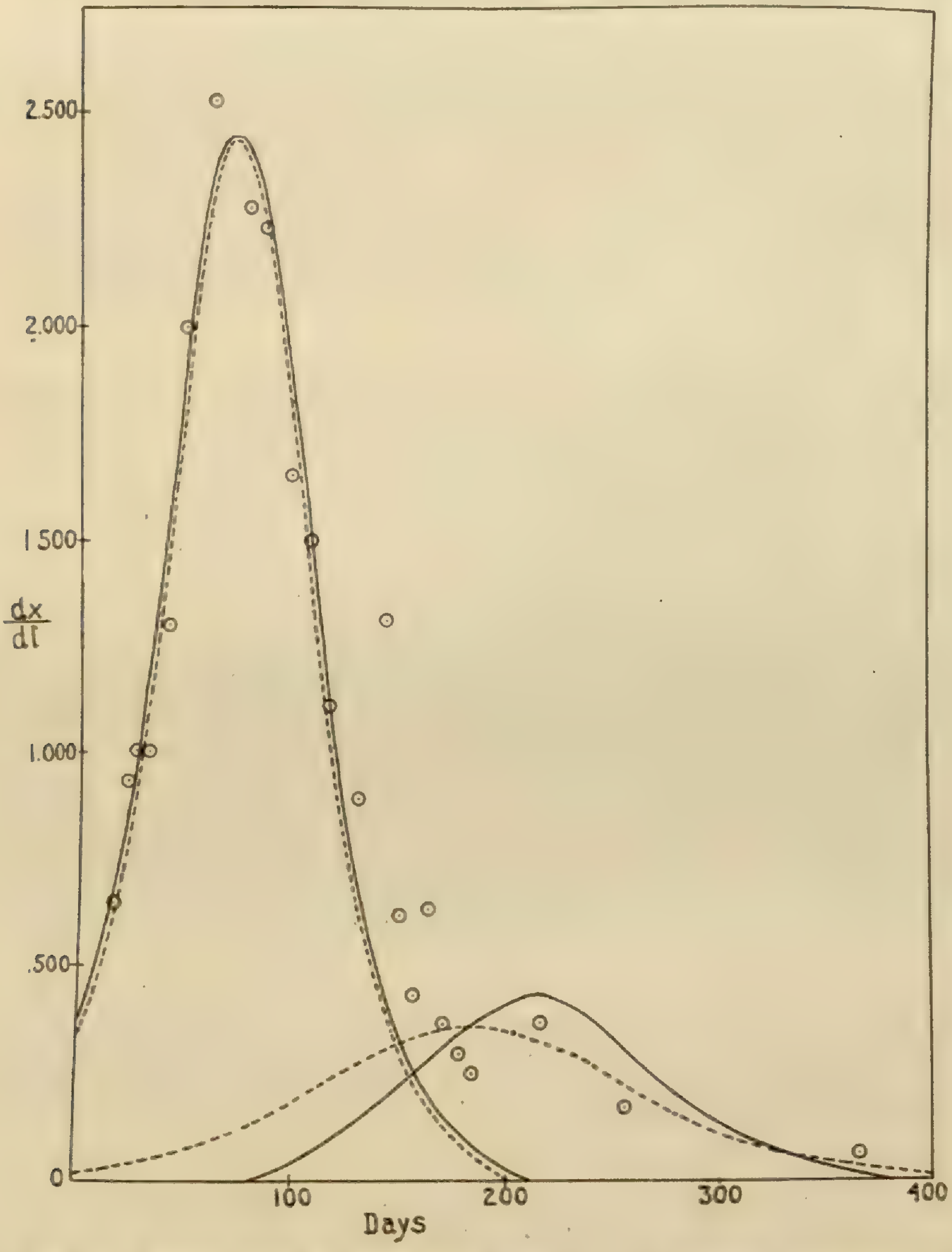

FIG. 6. Growth rates of male white rats. . . . . , observed increments of rats on adequate diet; ——, calculated values of dx/dt for rats on adequate diets; —— calculated values of dx/dt for rat recovering from a suppression of 370 days.

From birth to the 73d day the rate rose rapidly to its maximum, then fell to zero value of dx/dt at about the 215th day.

Before the first growth cycle was ended the second cycle had begun. The computed values show that this second cycle began about the 80th day of postnatal life, reached

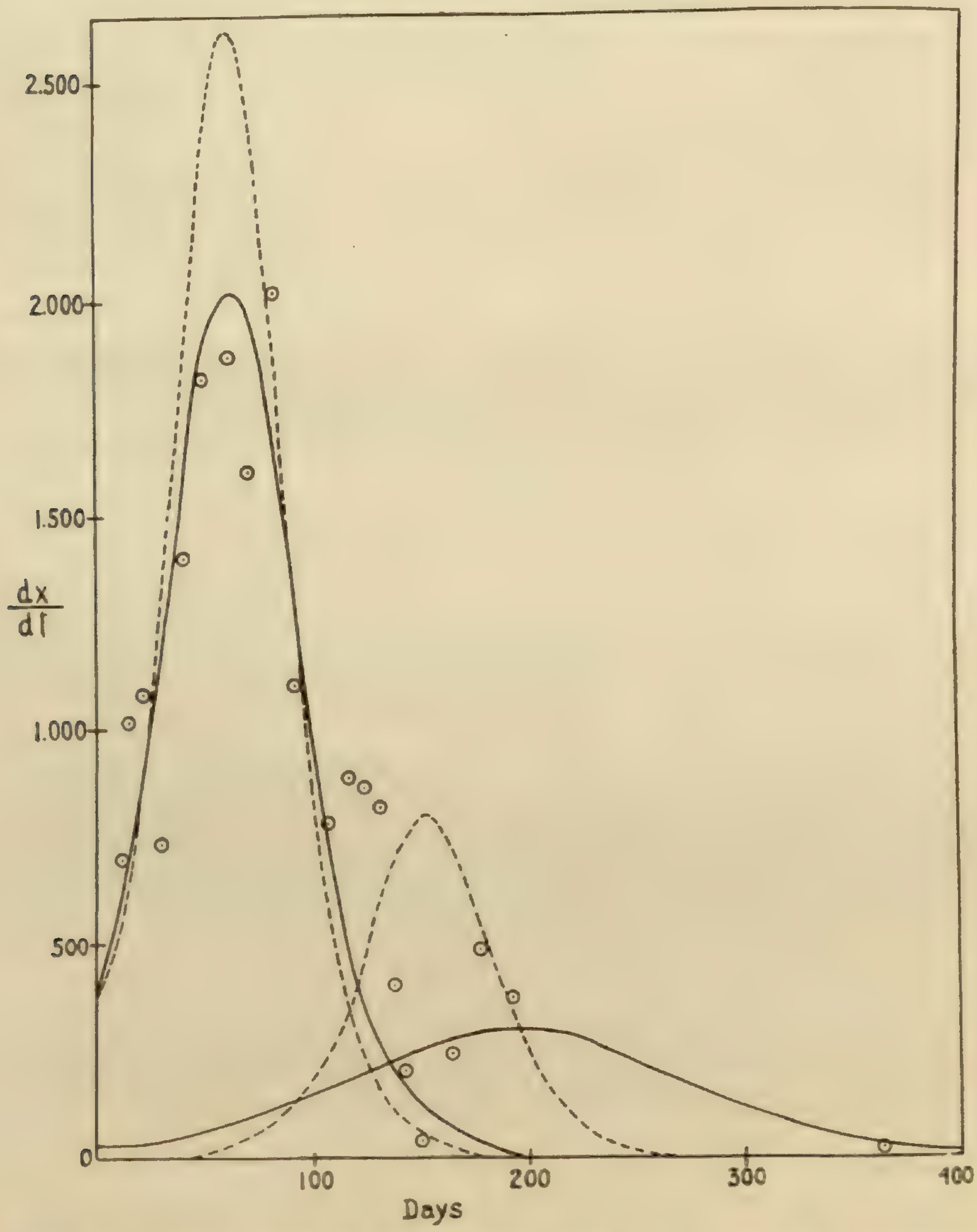

FIG. 7. Growth rates of female white rats. . . . . , observed increments of rats on adequate diet; ——, calculated values of dx/dt for rats on adequate diet , —— calculated values of dx/dt for rat recovering from a suppression of 500 days.

a maximum about the 213th day and ceased shortly before the 400th day. Since the value of $kx(a-x)$ approaches zero when x is very small and again when x is nearly

as large as a, it is possible to trace the curve in both directions until it reaches zero values of dx/dt. The actual values of dx/dt in the second cycle are much smaller than those in the first cycle.

Attention must be drawn to the way in which these two curves overlap. It will be noted that the second cycle of growth began shortly after the rate of the first cycle began to decline and that the first cycle continued to about the time of maximum rate in the second cycle. The true curve of the growth rate of these animals is, therefore, the arithmetical sum of the values of dx/dt for the various values of t. This is shown in Fig. 6 and agrees very well with the observed weekly increments.

The curves in Fig. 6 were obtained by plotting dx/dt as ordinate and t as abscissa.

If we let $dx/dt = z$, we may write

$$z = kx(a - x),$$

which when differentiated becomes

$$\frac{dz}{dx} = ak - 2kx.$$

If the right-hand member of this equation be equated to zero it will give the values of x for which z is either maximum or minimum.

Let $ak - 2kx = 0$; then

$$2kx = ak,$$
$$x = a/2.$$

Therefore the rate, z, is either maximum or minimum when $x = a/2$. To find whether z is maximum or minimum it is only necessary to get the second differential of the above equation

$$\frac{d^2z}{dx^2} = -2k.$$

Since this quantity has a negative sign z $(= dx/dt)$ is a maximum when $x = a/2$. In other words the rate of growth is a maximum when the cycle has reached a stage at which the weight of the animal is half the weight it attains at the end of that cycle. When $x < (a/2)$ the curve rises and when $x > (a/2)$ the curve falls.

The rate of growth of an animal recovering from an initial period of suppression may be studied in comparison with that of a rat on adequate diet. Take the case of rat No. 1012 (male). The computed values of dx/dt have been plotted in Fig. 6. The time at which dx/dt was a maximum in the first cycle was made to coincide with the maximum for the first cycle of the rats on adequate diet. This arrangement was adopted to facilitate comparison.

The curve for the first cycle as plotted in Fig. 6 is a very fair duplicate of the curve for the same cycle of growth for the rats on adequate diets. There is more difference in the case of the second cycle. The curve for rat No. 1012 has a maximum which is lower and occurs somewhat nearer the dx/dt axis than that of the other class of animals. In other words, the increased weight due to formation of fat in this animal began relatively earlier than in animals on adequate diets. In the main, however, there are no striking differences between the relative growth rates in the two cases, except that their maxima are nearer together.

The same sort of computations have been made for the growth of female rats and they are shown graphically in Fig. 7. An extended discussion of them is unnecessary, as it would be in many respects a mere repetition of what has been said. A comparison of the curves of female rats on adequate diet with those of rat No. 2033 shows (*a*) that the rate of resumed growth was faster in the second cycle, (*b*) that the second cycle was of shorter duration, and (*c*) that the maximum of the second cycle lay closer to that of the first cycle than in the case of rats on adequate diet.

In view of the fact that rats recovering from initial suppression reach mature weight more quickly than animals fed on adequate diets, it is somewhat surprising to find such a close similarity in the values of dx/dt for the same time intervals. One might expect that the curves for the recovering animals should be higher and steeper.

I believe that the reason for the quicker growth in the recovering animals lies, not in a faster growth rate in the cycle, but in the shorter time between the maxima of the two cycles. In other words, the final weight is reached more quickly because the second cycle of growth commences relatively earlier and is added to the first cycle. Unfortunately, the weights of recovering animals were not taken at sufficiently frequent intervals to afford data upon the actual rates of growth in this class.

If we assume that growth is the result of a catalyst acting upon a substrate, it seems that we have a key to the explanation of what is observed. The catalyst of the first cycle was produced in the pre-natal stages. Although there was no appropriate substrate available in the starved animals, the catalyst did not disappear. When an appropriate substrate was given, this catalyst acted upon it, producing a cycle of growth essentially equivalent to that shown by animals fed on adequate diets. This may mean that the catalyst persisted unimpaired until it was destroyed in the course of the reaction. The catalyst responsible for the second cycle likewise appeared and induced the formation of fat. If the second catalyst is in some way dependent upon a time factor for its formation (or activation) it is plain that it should show its activity relatively earlier in the case of animals recovering from a long period of initial suppression, because of a *quasi* cumulative age effect. The effect of this would be what we have seen to happen, viz., a crowding of the cycles nearer together.

The writer is fully aware of the hazards encountered in attempting to represent so complex a reaction as growth by a simple formula. The phenomena of growth appear, however, to be coordinated into a single self-consistent process, in which many chemical and physical factors are combined. The possibility of expressing growth by a simple formula showing that an increase in mass is definitely related with a function of time ought to lead to considerations of a fundamental nature.

D. Summary

1. The growth of white rats during the first year shows two cycles, and each cycle follows the course of an autocatalytic reaction. The first cycle covers the period in which the skeleton rapidly increases in size; the second covers the period in which there is a production and deposition of fat.

2. The equations for the growth of the two sexes differ in their constants, but each expresses the course of an autocatalytic reaction.

3. The earlier period of stunting did not prevent the animals from attaining the full weight characteristic of their sex after having an adequate diet. An equivalent gain in weight was made more quickly in the animals recovering from suppression than in animals on adequate diet.

In other words the animals grew somewhat more rapidly during their period of recovery.

4. The growth of white rats recovering from a long period of suppression follows the curve of autocatalysis, though a portion of the first cycle has been run during the long period of suppression.

5. The differential equations expressing the growth rates show that the two cycles overlap to some extent. The sums of the overlapping values approximate closely the observed increments. The second cycle of growth of rats recovering from starvation began and reached its maximum relatively earlier than in the case of rats on adequate diet.

6. There is evidence for the idea that each cycle of growth in this case had its specific catalyst and that the potential activity of the catalyst was not impaired by long periods of inadequate nutrition.

SHORTER ARTICLES AND DISCUSSION

ESTIMATING THE NUMBER OF GENETIC FACTORS CONCERNED IN BLENDING INHERITANCE

IN *Science* for July 29, 1921, Dr. W. E. Castle develops a "method of estimating the number of genetic factors concerned in cases of blending inheritance," which appears so simple, so attractively cogent, and so usable, that it is to be feared that the erroneous assumptions upon which it is based may be given less consideration than they merit.

About seven years ago,[1] I took some pains to point out certain fallacies which had crept into genetical literature through the tacit (or express) assumption that the several factors affecting the size of an organism or its parts, or the intensity of development of any character, are similar to each other in kind and equal in effectiveness. It has seemed to me that since that time there has been marked improvement in the literature dealing with this particular phase of genetical phenomena—whether in response to my paper or through independent following out of the simple logic of the case, it matters not. It comes as a distinct shock, therefore, to see the sudden reversion in Dr. Castle's paper to a supposedly outgrown and abandoned conception.

Dr. Castle has probably forgotten the bearing of my paper, though he commended it very highly in a letter written at the time of its publication; for so far as I can now recall he has never referred to it in any of his frequent papers, published since that time, on subjects involving the multiple factor hypothesis as an explanation of blended inheritance and the modification of a so-called "unit-character." This omission has been the more interesting because my paper even gave the precise interpretation of his hooded-rat case, which has been very lately espoused by him.[2]

Referring to the particular question now under discussion, my paper said:

> Attempts to determine how many plural determiners for any quantitative character are involved in a particular cross are as yet premature. Such attempts are based on the unproven hypothesis

[1] "Duplicate Genes for Capsule-form in *Bursa bursa-pastoris*," *Zeitschr. f. indukt. Abstamm. u. Vererb.*, 12: 97–149. 1914.

[2] Castle, W. E., "Piebald Rats and the Theory of Genes," *Proc. Nation. Acad. Sci.* [*U. S. Amer.*], 5: 126–130, 1 fig., April, 1919.

that the range of variability in F_2 equals the combined ranges of P_1 and F_1 generations, and the unwarranted assumption that the different plural determiners are essentially equal in effect.

What was premature seven years ago might be conceivably no longer premature, of course, but since the basis upon which Dr. Castle now proposes to estimate the number of such plural factors in specific crosses involves the same fallacies which made previous attempts untenable, it is needful to reiterate that nothing in the evidence justifies the belief that this new plan will give any sort of approximation to the actual facts. Nevertheless, the method proposed by Castle may be expected to have a certain amount of interest as representing a limiting case.

It is absurd to suppose that the height of a man will be as much affected, severally, by the factors which increase the thickness of the scalp, as by those which affect the length of the long bones of the legs; or that factors which produce changes in the number of internodes of a plant will generally add severally the same increment to the stature of the plant as will factors which increase the length of some or all of the internodes. Castle recognizes this weakness, but seeks to minimize its importance and declares that "no other assumption will permit of a general treatment of blending inheritance." He means, of course, merely that on no other basis can a generalized mathematical scheme be developed such as that which he has here presented.

But even if we allow such assumption of equivalence of genetic factors to pass on the ground of mathematical expediency, there are several other conditions involved in Castle's scheme which are equally unwarranted and which will profoundly affect the validity of the conclusions arrived at.

For example, the two strains mated together are supposed to stand at the two extremes of the total potential genetic variability in their progeny and all of the determiners are assumed to be additive in their effect, so that if we let the factors be represented in the usual manner by letters, the lesser parent must have the formula *XXaabbccddeeff* . . . , while the larger parent occupies the other extreme *XXAABBCCDDEEFF* . . . . It need scarcely be pointed out that while such a situation might be realized in some specific case it could not be generally true, and the greater the number of factors involved in any specific cross, the less likely would it be that the larger parent would possess them all and the lesser parent none. In my paper, referred to above, I said in regard to this point (p. 132):

Nilsson-Ehle (1911) has described a case in which the range of variation in the length of heads of wheat in the F_2 considerably exceeded the combined ranges of the two parents. Hayes (1912) has found a similar case in the number of leaves in tobacco, and Emerson and East (1913) have seen the same phenomenon in the length of internode and total length of stalks in maize. It seems probable that such transgressive variation may be the rule rather than the exception when very complex characters are investigated; for it is hardly to be expected that a large number of plural determiners affecting such a character shall all act in the same direction or that the parent having the highest development of the given character shall generally contain all the genes which the other chosen parent possesses. Whenever such transgressive variability is producible by the genotypic recombinations of parental characters, the frequency with which F_2 individuals simulate either parent gives no clue to the total number of plural determiners which have been brought together, with respect to any character under consideration.

We might even derive a mathematical expression for the probability that the parents would stand at the extremes of total genetic variability, by assuming that the two parental types are taken at random. This would be perhaps a fair assumption, since the number of factors can not be determined by inspection. There would then be, if we let n represent the number of factors involved in the cross, $n!/2$ ways in which the event in question can not happen and only one way in which it can happen; hence the probability that all the factors would be present in the larger parent and absent in the lesser parent would be as 1 to $n!/2$. In the specific case of East and Emerson's corn, cited by Castle as having about 15 factorial differences, ($n=15$), there would be, therefore, 633,477,184,000 chances to one against all of these size-modifying factors being present in the larger parent and absent in the smaller parent. When we let $n=50$ or 150, to agree with the numbers indicated for the rabbit crosses, the chances become practically infinitesimal and we must fairly conclude that it has never happened, and never will happen, that a cross involving so many independent size differences has been made, or will be made, between individuals standing at the opposite extremes of the total potential genetic variability.

The remark made in my paper on duplicate genes, regarding the inadequacy of the frequency with which parental types are duplicated in the F_2 as an indication of the number of factors involved, applies equally well to the validity of conclusions drawn from changes in the F_2 standard deviations. The

correctness of this conclusion will be made sufficiently obvious by consideration of a simple illustrative case: Let us assume that there are involved in a given pair-mating the six duplicate size-factors, *AABBCCDDEEFF*, and their absences or corresponding recessives, *aabbccddeeff*. The relation of the F_1 and F_2 variability will be exactly the same no matter which of the four following types of mating is made: (*a*) *aabbccddeeff* $\times$ *AABBCCDDEEFF* (difference between parents, 12 units); (*b*) *AAbbccddeeff* $\times$ *aaBBCCDDEEFF* (parental difference, 8 units); (*c*) *AABBccddeeff* $\times$ *aabbCCDDEEFF* (parental difference, 4 units) or (*d*) *AABBCCddeeff* $\times$ *aabbccDDEEFF* (parental difference, zero). Neither Castle's original scheme nor Wright's suggested modification[3] of it can be true, at one and the same time, for more than one of these types of mating and they have been specifically designed only for the first-mentioned type (*a*); but as we have seen in a previous paragraph, if the number of factors involved is large, the number of matings of type (*a*) is almost infinitesimal in comparison with the number of matings of the other types, (*b*) + (*c*) + (*d*) + (*e*) + . . . to *n* terms.

It is not necessary, however, to suppose that all factors which affect a blending character are additive. Indeed, it is quite certain that they are not, and that some factors act in a negative direction and others in a positive direction. Inhibiting and depressing factors have been fully demonstrated, as may be exemplified by such classic cases as the inhibitors of horn production in cattle and sheep; the inhibitor of indeterminate growth in the tail of the Japanese long-tailed fowl; the condensation factor characteristic of the *compactum* type of wheat; and a series of dominant depressing factors which Davenport's[4] studies have made probable in the case of human statures. The fact that Castle himself was able to make progress in the minus direction in his selection experiments suggests the probable accumulation of factors acting in a negative direction, though in this case the evidence is not decisive, as the same effect would have been secured by the gradual elimination of factors acting in a positive direction. It seems to me that the combined action of plus-act-

[3] Castle, W. E., "An Improved Method of Estimating the Number of Genetic Factors Concerned in Cases of Blending Inheritance," *Science*, 44: 223, Sept. 9, 1921.

[4] Davenport, C. B., "Inheritance of Stature," *Genetics*, 2: 313–389. July, 1917.

ing and minus-acting growth factors gives a very true, though somewhat formal conception of the general situation in all organized beings;—the interplay of growth-promoting and growth-inhibiting factors may be thought of, in a figurative sense, as forming, within the limits of fluctuating variation, a sort of elastic "mold" into which any organism, whether plant or animal, develops, and which gives it its wonderful specificity of form and size.

But on the basis of this conception of factors, acting, some in a positive and some in a negative direction, the combined action of the negative group exactly balancing the combined action of the positive group, and jointly determining the mean size or the average condition with respect to any blending character which may be under consideration, it becomes unnecessary to assume the absence of dominance. I have been teaching my students for the past six years that the postulation of lack of dominance which has always been made the basis of the multiple-factor interpretation of inheritance of size or of other blending characters is wholly unnecessary and that those who have discussed this type of inheritance have been led to place an altogether unnatural and unwarranted stress on the occasional occurrence of incomplete dominance in other cases.

But whether any or all of the size-factors are dominant or not materially affects the amount of change which they effect in the value of the F_2 standard deviation, and must correspondingly change the estimate of the number of factors involved when that estimate is based on the value of these F_2 standard deviations. As a simple example, I may cite the hypothetical illustration given in my 1914 paper (p. 129), referred to above:

> Thus, if a plant possessing a partial inhibitor or reducer of internode-number be crossed with another plant having a stimulator for internode length, all the other genes being the same in the two cases, the height of the F_1 plants would be intermediate between the heights of the parents, with variability due alone to fluctuation, as it is in the homozygous parents. The F_2 would show increased variability, and *this increase would appear greater if the two differentiating genes were dominant, than if dominance were absent.*[5]

To illustrate this fact further, let us assume that the six size-modifying factors which differentiate two mates are *AABBCC*, acting in the plus-direction, and *DDEEFF*, acting

[5] Not italicized in the original.

in the minus-direction, and that the effect of each factor pair is represented by 2 units, thus making up the total of 12 units arbitrarily chosen by Castle. If the various permutations are worked out, the F_2 series of frequencies is found to be as follows:

TABLE I

DISTRIBUTION INTO SIZE-CLASSES RESULTING FROM THE INTERPLAY OF SIX FACTORS, 3 POSITIVE AND 3 NEGATIVE, WHEN DOMINANCE IS WANTING AND WHEN DOMINANCE IS COMPLETE.

Condition as to Dominance	Class Values and Frequencies												
	−6	−5	−4	−3	−2	−1	0	1	2	3	4	5	6
No dominance	1	12	66	220	495	792	924	792	495	220	66	12	1
Dominance complete	27		270		981		1,540		981		270		27

These frequencies are exhibited graphically in Fig. 1, the curve produced by dominant factors being reduced to the same area as the curve of no dominance by dividing each frequency by 2. It is thus seen clearly that whether dominance is present or absent, the resulting curve is of the same general type.

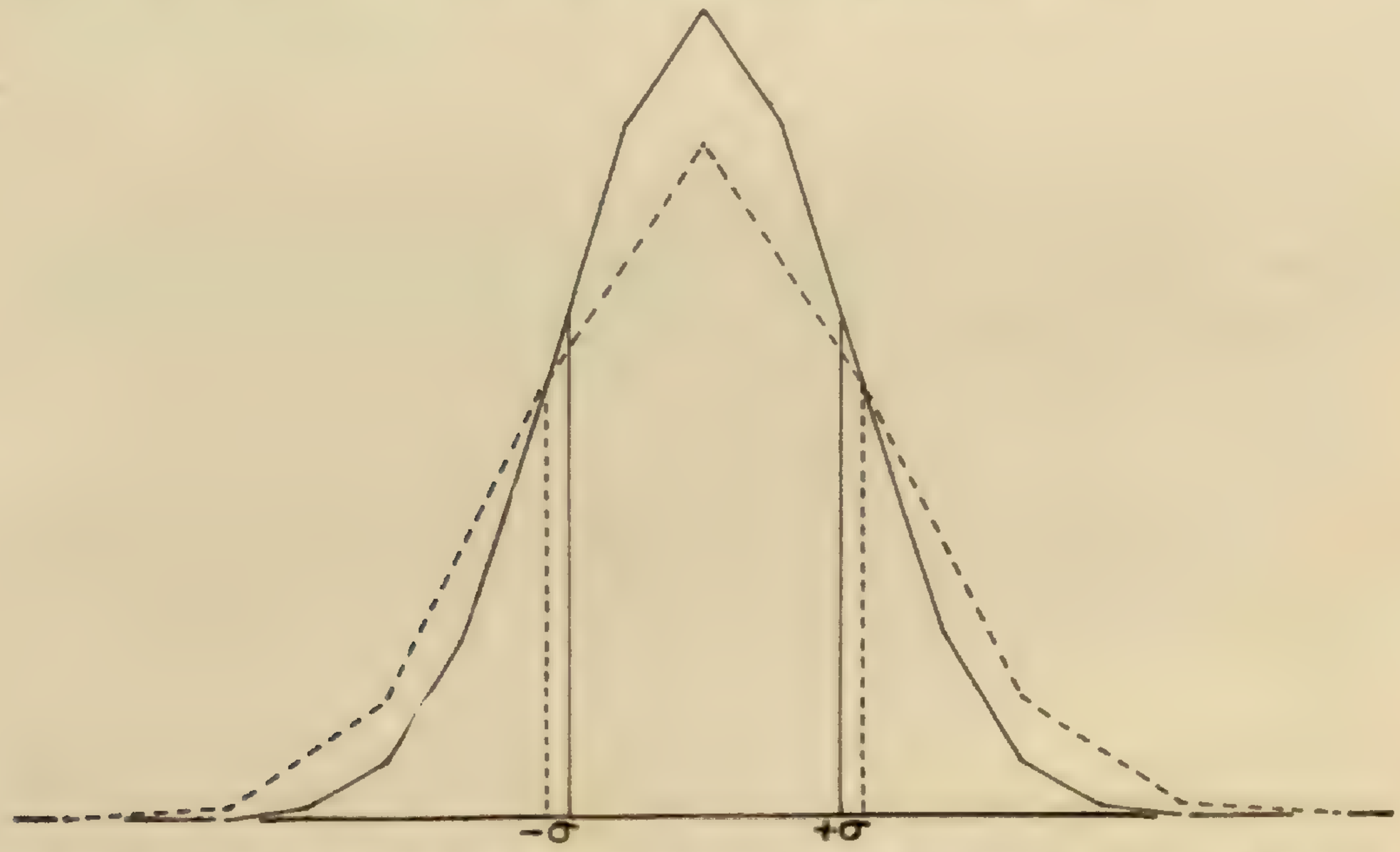

FIG. 1. The variation produced by six equivalent factors, three acting in plus direction and three in minus direction. The unbroken curve shows the result when dominance is wanting. Dotted curve shows the effect of complete dominance in all six factors.

The standard deviation of the curve with no dominance is $\sqrt{3} = 14.43$ per cent., as Castle has stated, but when all of the

six factors are completely dominant the standard deviation is $\sqrt{4.5}$, or 17.67 per cent., which is the percentage corresponding to 4 factors as given in Castle's Table II. In other words, six factors with dominance produce as great an increase in the F_2 variability as four factors would produce if none of them exhibited dominance.

It can be shown in the same way, by means of a simple test case, that Castle is entirely in error in saying that "if one factor really has an influence greatly superior to that of other factors in a case of blending inheritance, this will be seen in the production of asymmetrical or multimodal variation polygons in F_1 and F_2." The consequence he mentions would follow only in case the factor having the greater influence happened to be dominant, for in the absence of dominance each factor enters into combination with every other factor in a 1 : 2 : 1 series of intensities which gives the probable-error type of distributions. Thus, if we assume that four factor pairs *AABBCCDD* differentiate two chosen mates with respect to some blending character, and that three of these factor pairs, *AABBCC*, each acts in a plus direction with a value of 2 units, and the fourth factor pair, *DD*, acts in a minus direction with a value of 6 units, the F_1 will be intermediate as before while F_2 will give the series of frequencies shown in Table II.

TABLE II

DISTRIBUTION INTO SIZE-CLASSES RESULTING FROM THE INTERACTION OF THREE FACTOR PAIRS ACTING IN THE PLUS DIRECTION, EACH ADDING TWO UNITS, AND A FOURTH FACTOR PAIR ACTING IN THE MINUS DIRECTION AND SUBTRACTING 6 UNITS

Condition as to Dominance	Class Values and Frequencies												
	−6	−5	−4	−3	−2	−1	0	1	2	3	4	5	6
No dominance	1	6	15	22	27	36	42	36	27	22	15	6	1
Dominance complete	3		27		81		82		9		27		27

It will be noted that while the curve of no dominance in this case is not quite a typical probable-error curve, it is nevertheless perfectly symmetrical and the deviations from the typical curve are such that they would never be detected when the several classes are modified by concurrent fluctuations from environmental causes.

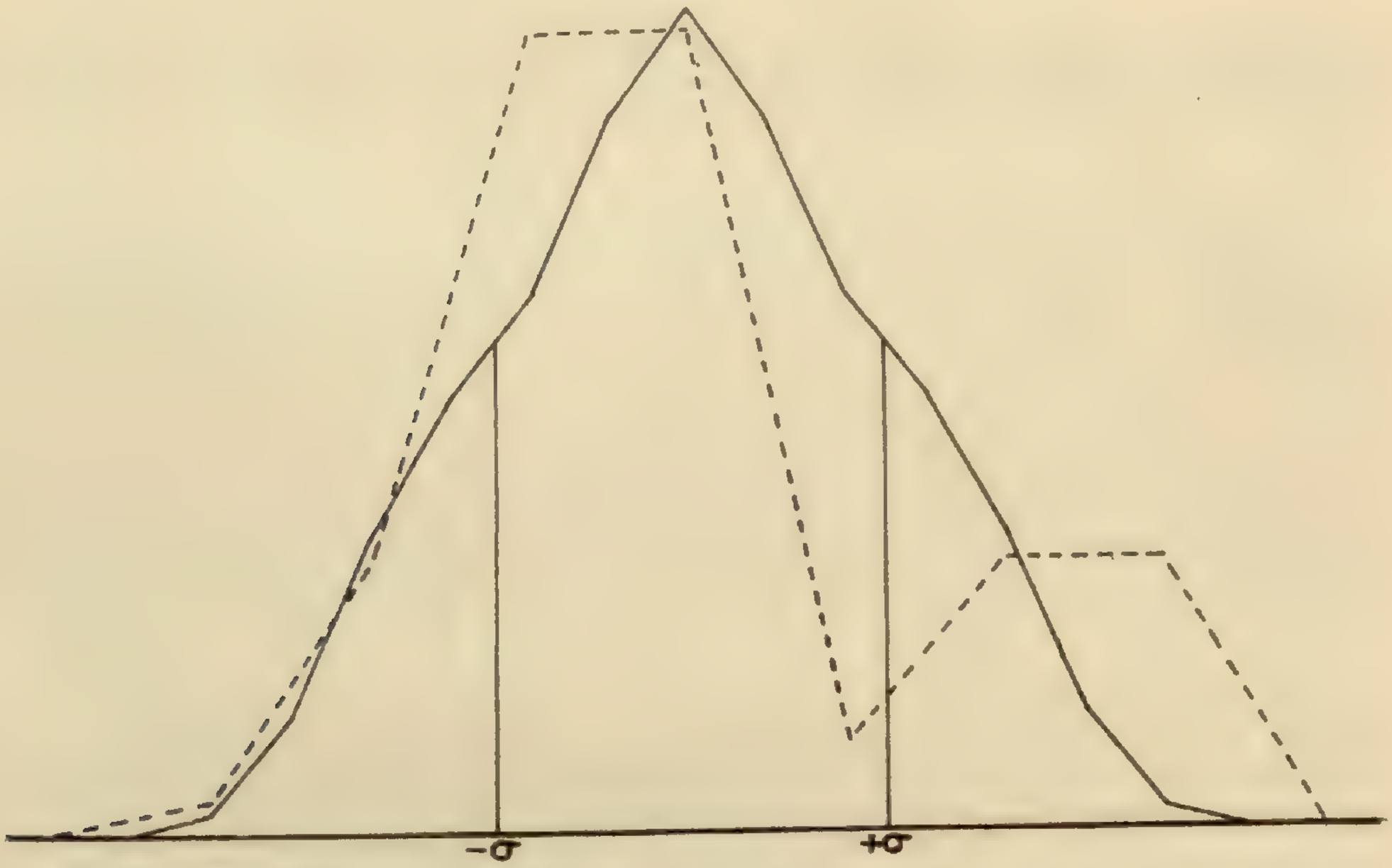

FIG. 2. Variation curve for four factors, three acting in plus direction, each with an effect of two units, and one acting in minus direction, with an effect equal to six units. Unbroken curve shows no dominance in any factor; dotted curve shows effect of complete dominance in all four factors.

The standard deviation of this series of values is $\sqrt{6}$, or the same as would be produced by 3 factors if all were of equal value, as shown in Castle's table. This curve is shown graphically in Fig. 2 together with the corresponding curve for factors weighted in the same manner, but exhibiting dominance. Such an irregular, multimodal curve as the latter would be easily detected if there were no fluctuating variations, but Castle apparently overlooks the effectiveness of fluctuating variations in obscuring the details of the underlying curve of genotypic variability. When he interpolates between the standard sizes of two breeds of rabbits 50 to 150 centers of genetic stability, he should take into account that the fluctuations about each of these centers will be sufficient to scatter the individuals which belong in any one class over quite a considerable number of other classes, thus filling up the gaps and hiding a great deal of putative multimodality in the genotypic curve, which would result from inequalities in the relative effectiveness of the several plural factors involved in any case of blending inheritance. Castle apparently thinks that this confusing concurrence of genetic and fluctuating variation could be dissolved to a certain extent by rearing "adequate numbers" in F_1 and F_2, but it must not be forgotten that the masking effect of fluctuations advances *pari passu* with the increase in the size of the population. Only the irregularities due

to too small size of the random sample could be eliminated by the rearing of larger numbers; the effects of the interplay of numerous environmental factors could not be thus eliminated.

From the foregoing considerations it must be clear that Castle is altogether too sanguine as to the value of his method when he says:

It is perhaps not to be expected that results more than approximately correct would be given by this method, *unless fairly large numbers of both F_1 and F_2 individuals have been studied.*[6]

I believe the conclusion is justified that even a "fairly large" number of individuals of the F_1 and F_2 could not be expected to give correct estimates of the number of factors interacting in any case. As between the method of Castle in estimating the number of hypothetical duplicate factors operating in any case on the basis of the change they produce in F_2 standard deviations, and the method of Punnett, of formulating a genotypic situation on the basis of a small number of definitely weighted factors, I am convinced that the latter method is much to be preferred, even though it does not lend itself readily to a "general treatment of blending inheritance."

GEORGE H. SHULL

PRINCETON UNIVERSITY

DR. SHULL has kindly sent me his manuscript in advance of its publication and generously asks me to comment on it, an invitation which I gladly accept.

The difficulties which he thinks might be encountered in applying the method, which I have suggested, for estimating the number of genetic factors involved in cases of blending inheritance, seem to be essentially these.

1. The possible unequal influence of the several factors which are responsible for a case of blending inheritance, making it difficult to estimate their number from the total effect observed.

2. The possibility that some of the factors may be dominant in character and others not.

3. The possibility that some may be positive in action and others negative or inhibitive.

I think that these difficulties arise chiefly from the attempt of Dr. Shull to extend the application of the method beyond the field for which it was proposed. Cases of dominance or of trans-

[6] Italics are mine.

gressive variation in F_2 would not come within the scope of blending inheritance as I defined it, when proposing the method, but only cases in which both F_1 and F_2 are intermediate between the parent races. But Dr. Shull maintains that some dominant *factors* may be involved even where dominant characters are not in evidence. This may be admitted as a possibility even though we have no evidence for it. What of it? Dr. Shull formulates a test case, the strongest one imaginable, in which *all* (six) factors affecting a size character are "completely dominant" and finds that in this case the method which I suggested would indicate a smaller number of factors than the true one, or four instead of six. If this is the maximum error to be anticipated when *all* factors are completely dominant, and it is really doubtful whether *any* factors are dominant in the case of blending characters, the possibility need not give us great concern. Further, if all factors involved in producing a character are "completely dominant," how can the character itself keep from being dominant? And if it is dominant, the case will be automatically removed from the field of blending inheritance. Shull seeks to avoid the difficulty in his hypothetical case by putting three dominant factors in one parent race and three in the other, but this arrangement, by recombination of factors, in F_2 would result in segregation of the parental types or in transgressive variation, either of which events would remove the case from the category of blending inheritance as I have defined it.

The supposed difficulty, that some factors involved in the production of blending characters may be positive in action while others are negative, is purely formal. With three positive and three negative factors, in his hypothetical case, Shull comes out with identically the same distribution in thirteen size classes that I calculated for the same number of factors all positive, both of us assuming no dominance to occur.

It remains to deal with the first-mentioned difficulty, the possible unequal influence of the several factors assumed to occur in blending inheritance. I had anticipated this difficulty in my first paper, but had assumed that, "if one factor really has an influence greatly superior to that of other factors in a case of blending inheritance, this will be seen in the production of asymmetrical or multi-modal variation polygons in F_1 and F_2."[1] Shull challenges this statement and artfully constructs a case to

[1] I should have limited the statement to F_2.

disprove it in which the influence of one factor exactly equals and negatives that of three other factors. In this case he finds that the variation curve is symmetrical when no dominance occurs, but asymmetrical and bimodal otherwise. Had the hypothetical factors been less carefully weighted by Shull, he would not so easily produce a symmetrical F_2 curve. Consider an actual case in which a single factor of superior influence occurs and yet in which there is no dominance, that of the blue Andalusian fowl. Black mated with splashed white produces blue in F_1, an apparent blending. Yet F_2 falls, as every one admits, into three distinct classes notwithstanding the occurrence of one or more modifying or inhibiting factors affecting the result in a minor degree (Lippincott). "Fluctuating variation" does not here obscure segregation, as Shull assumes would be true in hypothetical cases of blending inheritance in which factors of very unequal influence occur, even when large numbers are studied. Now I should not class the case of the Andalusian fowl as blending inheritance, but I think it may serve to show that Shull's objection is not well founded, in accordance with which he assumes that a factor of major influence will not be readily detected, even when it is operating in conjunction with minor modifying factors.

W. E. CASTLE

TO THE EDITOR OF THE AMERICAN NATURALIST: The foregoing article and rejoinder are submitted for publication in their original form. I do not think that Castle's "comment" adequately meets the several difficulties which I have pointed out, and he presents no considerations which seem to me to warrant a modification of the statements I have made. Others who are interested in the topic under discussion may be depended upon to recognize the validity or non-validity of any of the propositions made by either of us. It should be said, however, that there was nothing "artful" in my choice of an illustrative case to show that inequalities in the effectiveness of the several genes do not necessarily produce asymmetrical and multi-modal variation-polygons when dominance is not present. The case could have been made still more striking by using a larger number of factors, but the additional labor required did not seem necessary. Obviously Castle has not tried out cases in which the factors are weighted differently from the weightings I assigned to them,

or he would not say that in that case I "would not so easily produce a symmetrical F_2 curve."

That he has not assimilated the significance of the inter-play of plus-acting and minus-acting factors is shown by his question:

If all factors involved in producing a character are "completely dominant," how can the character itself keep from being completely dominant?

The curves in my Fig. 1 are an adequate answer to this question.

Finally, it seems hardly necessary to point out the inadequacy of the case of the Blue Andalusian fowl, as a proof that inequalities in genotypic variation are not masked by fluctations when the amount of fluctuating variation is large in comparison with the distances between the centers of genetic stability which are determined by the numerous factor combinations putatively involved in the several examples cited in Castle's original paper.

GEO. H. SHULL

GENETIC TERMINOLOGY

THE genetic terms recently proposed by G. H. Shull[1] seem to supply a real need. Their general use would certainly tend to reduce both the danger of ambiguity and the need for cumbersome descriptive phrases. Probably a few additional numerical terms, such as *dizygous* and *trizygous*,[2] would also be useful. Some existing equivalents have an obvious disadvantage with respect to compounding; the compound *trichromosomal*, for example, could not replace *trizygous* ("dependent on three *pairs* of chromosomes"). *Monozygous* and *pleiozygous*, although often interchangeable with *linked* and *unlinked*, should be useful; the latter, for example, to characterize genes that are located in several pairs of chromosomes but are not all necessarily unlinked with each other. These words are so interrelated among themselves, and so closely related to terms in general use, that all their advantages can be realized with little effort.

Shull suggests that it is time "to abandon the use of 'Mendelian' and 'non-Mendelian' as definite categories, and to adopt

[1] Shull, George H., "Mendelian or Non-Mendelian?" *Science*, N. S., 54: 213–216. Sept. 9, 1921.

[2] For the Greek numeral prefixes see Blakeslee, Albert F., "Types of Mutations and their Possible Significance in Evolution," AM. NATURALIST, 55: 254–267. 1921.

other terms which will have greater precision of meaning.'' Let us accept his timely proposal, which obviously applies especially to the more technical and precise terminology of genetics. With his new terms available, we may safely relegate the older ones, aside from historical references, to the more popular language of science.

Shull uses the older words to illustrate the application of his proposed terminology, but he does not specifically discuss their future delimitation in case they still retain a certain usefulness. Their future, I believe, deserves consideration. It seems certain that they will remain familiar words because of their historical value, in relation both to Mendel's work and to its earlier extension. Doubtless they will long be especially useful in the more popular presentation of genetic topics, to obviate burdensome use of more precise but more formidable expressions.

Historically, it is plain that the meaning of *Mendelian* has very largely kept pace with the widening conception of the fundamental applicability of Mendel's theory, although often, as Shull states, with the addition of qualifying expressions. When this widening process reaches the farthest point of practical usefulness, it leads to a broad definition of *Mendelism* which, I believe, deserves general acceptance.[3] It furnishes, for example, a convenient and familiar popular equivalent of *zeuxis* for the characterization of ''chromosomal heredity,'' at least so far as the inheritance phenomena of sexual reproduction are concerned. All other senses of *Mendelian* seem to require more technical detail in definition, or to be otherwise less useful for the purpose in question.

Again, the most significant conflicts of ''Mendelism'' with its critics have raged along a line of demarkation essentially corresponding to the broader definition. ''Mendelians'' once encountered frequent denials of the completeness and the generality of segregation, and frequent assertions that new somatic ratios implied other modes of inheritance of equal significance with Mendel's. The triumph of the chromosome theory has been the definitive establishment of the fundamental significance of ''Mendelian heredity.''

Finally, the broadest definition is fully justified logically, although it may not be superior in this respect to some other delimitations of the term. It may be held with good reason that

[3] Not forgetting, of course, that the older usage varies.

even linkage represents an *addition* to Mendel's genetic theory, rather than an *exception* to it. His scheme of independent separation and recombination of potentialities at gametogenesis is still adequate for the innumerable cases resembling his. Further, all genetic factors belong to theory rather than to observed fact, as do atoms and molecules. A gene is a *supposed* reality; it is something which many geneticists now *assume*, on the basis of evidence which they consider essentially conclusive, to be an *actual* part of a chromosome. The idea of lethal genes, therefore, or even that of the gene as a part of a chromosome, just as truly constitutes an addition to Mendel's genetic theory as a whole, as does the explanation of linkage ratios. The difference is, from this viewpoint, one of degree rather than of kind. If we admit some added hypotheses as Mendelian, why should we necessarily exclude any others which plainly relate to the same unified nuclear mechanism?

Even if we hold, as we may, that Mendel's theory has been revised as well as extended, its most fundamental feature, by present standards, is left unchanged. What, from our present viewpoint, is Mendel's most fundamental genetic conception? Is it not that of a *genetic shuffling*, a segregation and recombination, of genetic units which maintain their individuality throughout the processes of reproduction and of development?[4] Most usefully and even most commonly, it seems to me, *Mendelism* signifies *the general type or mode of inheritance* whose most fundamental principle of character distribution was discovered by Mendel; and this is "zeuxis," or chromosomal heredity, in sexual reproduction. This delimitation of *Mendelism* seems to me fully as good logically, better in accord with history, and much more promising of future usefulness in the field where the term is still needed, than any of the less inclusive senses in which it has been employed.

Dr. Shull says in correspondence, "I can see no objection to the *general non-technical use* of the words 'Mendelian' and 'Mendelism' in just the sense which you propose." And I believe that Morgan, East, Jones and Wright are far from being alone when they positively favor the broader definition.

[4] Bateson, W., "Mendel's Principles of Heredity." 1909. Cambridge Univ. Press. (See p. 13.)

Morgan, T. H., Sturtevant, A. H., Muller, H. J., and Bridges, C. B., "The Mechanism of Mendelian Heredity." 1915. New York, Henry Holt & Co. (See p. 1.)

Let us, then, take the course which is obviously more useful, and also honor the memory of the great pioneer of genetics, by applying his name to his great idea in all its later ramifications. But—wherever newer and more precise terms will better promote the science of genetics, let us be ready to use them. Shull's recent contribution to genetic terminology promises considerable and lasting usefulness.

HOWARD B. FROST
UNIVERSITY OF CALIFORNIA

IN connection with Dr. Shull's[1] interesting and important proposals concerning genetic nomenclature, attention should be called to a situation which neither these proposals nor the terminology in general use recognize. It is customary to refer to individuals carrying single X or Z chromosomes, as being heterozygous for sex-linked genes. For some time this has seemed to be ill-advised to the writer.

The situation prevailing in an XX or ZZ individual heterozygous for a sex-linked gene clearly differs from that of an XY (or XO), or a ZW individual in the vast majority of cases, though Schmidt's work on *Lebistes reticulatus*, to which Dr. Castle[2] recently called the attention of American workers, possibly indicates that for XY individuals it does not necessarily always differ. In the one case, usually there is a demonstrable allelomorph, not infrequently competitive enough in its expression to produce more or less of an intermediacy between the two homozygous forms. In the other, usually there is not.

The term heterozygous, as much as homozygous, indicates an allelomorphic pair, yet in XY and ZW individuals, with the one possible exception noted, a pair of sex-linked genes has not been demonstrated, and is clearly impossible for XO individuals. To all appearances the sex-linked genes in such individuals are without synaptic mates. They are therefore simplex but not heterozygous.

In order to recognize this situation, and in a measure describe it without using presence and absence terminology, and in harmony with the terms proposed by Dr. Shull, I should like to suggest the noun *hemizeuxis* (a half yoking) and the corresponding adjective *hemizygous* (half yoked). Should such

[1] Shull, Geo. H., 1921, *Science*, N. S., 54: 213–216.
[2] Castle, W. E., 1921, *Science*, N. S., 53: 339–342.

a suggestion prove acceptable there would be the three adjective series: homozygous, heterozygous, and hemizygous, referring to the three possible conditions with respect to any single gene, namely, "like mates," "differing mates," and "no mate."

The term might also be used in cases where a non-deficient chromosome is paired with a deficient one. The deficient individual would be hemizygous for the genes at those loci of the non-deficient chromosome which were involved in the deficiency of its mate.

WILLIAM A. LIPPINCOTT
KANSAS AGRICULTURAL EXPERIMENT STATION

CROSS-OVER VALUES IN THE FRUIT-FLY, DROSOPHILA AMPELOPHILA, WHEN THE LINKED FACTORS ENTER IN DIFFERENT WAYS

THE factors for bar, round, red and white eye are sex-linked, being located in the X-chromosome, as shown in inheritance. For example, when a bar-eyed male is mated to a normal female all the resulting male offspring (F1) are normal and all of the females bar eyed, as bar is dominant; that is, factors that occur in the X or first chromosome have a "criss cross" mode of inheritance following the distribution of the X-chromosomes, there being but one X-chromosome in the males and two in the females.

The relative positions of the linear series of factors in the X-chromosome of *Drosophila* have been determined by Morgan and Bridges (237 Carnegie Institution). Factors that lie near together in the chromosome are more likely to be transmitted in the same combinations to the gametes than those that lie far apart; that is, the strength of linkage depends on the distance apart of the factors. The failure to transmit the same combinations of factors that enter from the parents to all the offspring is due to a crossing-over of some of the factors. For example, a red bar-eyed fly, mated to a white round-eyed fly, give in the second generation (F2) white bar and red round-eyed flies, as well as flies like the original parents; that is, there has occurred a recombination of the factors due to crossing-over.

Are the cross-over values the same when the linked factors enter in different ways? My experiments performed in the University of Chicago Laboratories give data relative to this question. Using the presence and absence hypothesis, let (B) represent the factor for bar and (R) the factor for red, then let

(b) represent the absence of the factor for bar and (r) the absence of the factor for producing red-eyed flies. The factorial composition, then, of the red bar-eyed flies is (BR) and for white round-eyed flies (br). As both the factors for bar- and red-eyed flies are sex linked—that is, they occur in the X-chromosome, there being but one in the male and two in the female, as above mentioned. Then the male red bar-eyed flies are represented by (BR) and the female white round-eyed flies by (br—br).

Matings were made as follows:

(2 pairs) Red bar-eyed males (BR). White eyed females (br—br) F1 171 White round-eyed males (br). 184 Red bar females (BR—br).

	F2 Males.				F2 Females.			
	Red Bar.	White. Bar.	Red.	White.	Red Bar.	White Bar.	Red.	White.
(27.1)	50	37	32	50	49	31	42	34
" .2	26	17	30	31	38	25	27	40
" .3	39	32	29	42	40	21	33	30
" .4	26	15	27	17	28	12	29	17
" .5	53	26	55	41	51	27	35	29
" .6	32	11	23	26	43	17	27	30
	226 (BR)	138 (Br)	196 (Rb)	207 (br)	249 (BR br)	133 (Br br)	193 (Rb br)	180 (br br)

As white bar-eyed flies and red-eyed flies are the cross-over classes, then the percentage of crossing-over is equal to 660, divided by 1,522, and the quotient, multiplied by 100, giving 43.3 per cent.

I next extracted pure lines of white bar-eyed flies and red-eyed flies, using the white bar-eyed males and red round-eyed females in the matings. Their factorial composition being (Br) for the males and (Rb—Rb) for the females.

F1 99 red-eyed males (Rb) and 105 red bar-eyed females (Rb—Br) F2 (from about thirty matings from F1).

Males.				Females.	
Red Bar.	White Bar.	Red.	White.	Red Bar.	Red.
1127 (BR)	1265 (Br)	1456 (Rb)	1045 (br)	2658 (BR or else Rb) (Br Rb)	2606 (Rb or else Rb) (br Rb)

The cross-over classes are the red-bar and white-eyed male flies. The percentage of crossing-over being 2,172, divided by 4,893, and the quotient, multiplied by 100, which gives 44.4 per cent. The difference, then, in the cross-over values when the linked factors entered in different ways was but 1.1 per cent., which does not seem to be a signicant difference.

J. D. Ives.

ON COUNTING CHROMOSOMES IN POLLEN-MOTHER CELLS

The genetic study of hybrids between species with different chromosome numbers and of certain mutants requires the counting of many chromosome groups and raises the question of the best technique for the purpose. The staining qualities of aceto-carmine, which has long been used for preliminary work, especially by zoologists, are considerably improved by a trace of ferric salt. (Bolles Lee, in his well-known manual, gives formulæ for iron carmine; but this has no advantage for sections over iron hæmatoxylin.)

Iron Aceto-carmine 1.—Ordinary aceto-carmine is prepared by heating a 45 per cent. solution of glacial acetic acid to boiling with excess of powdered carmine, cooling and filtering. The young anthers are teased out with steel blades or needles in a drop of this until it changes slightly toward bluish red. An excess of iron spoils the preparation. Anther remains are removed, and a large thin coverglass (22 by 50 mm.) applied, using the minimum of liquid. The edges are sealed with vaseline. The preparation, if there is no excess of iron, may improve for a day or two.

Iron Aceto-carmine 2.—To a quantity of aceto-carmine a trace of a solution of ferric hydrate dissolved in 45 per cent. acetic acid is added until the liquid becomes bluish red, but no visible precipitate forms. An equal amount of ordinary aceto-carmine is then added. The anthers are teased out with nickel instruments. If the stain is too dark, more aceto-carmine is to be supplied. It may be diluted with 45 per cent. acetic.

Iron Aceto-carmine 3.—Anthers at the right stage are put into a mixture of 1 part of glacial acetic acid to 9 parts of absolute alcohol, to which sufficient solution of ferric hydrate in 45 per cent. acetic has been added to color the liquid brown (the amount

varies with different objects). After some days or weeks the anthers are teased out in ordinary aceto-carmine, avoiding the use of steel instruments.

The chromosomes are usually most accurately counted in the metaphase of the second division, in dicotyledons. When the preparation is a day or two old, the cytoplasm has swollen; and a slight tap on the thin coverglass above any particular cell will usually free the cytoplasm from the cell wall, and another tap flatten it out with its contained chromosomes.

Satisfactory results have been obtained by these methods during the past year with *Datura, Canna, Antirrhinum, Linaria, Brassica, Dahlia, Secale, Asparagus, Matthiola, Phaseolus, Stizolobium, Tradescantia, Hemerocallis, Iris, Gladiolus, Zea* and *Portulaca.* The methods failed with *Œnothera* and *Rhododendron.*

The second of the above methods will probably be of the widest applicability. The preparations will keep for a week or more, if an excess of stain and of iron are avoided. The method is quicker for counting chromosomes than staining sections with iron hæmatoxylin, and in favorable cases the results may be more certain. Thus in good preparations of *Datura* over a thousand pollen-mother cells are scattered singly on one slide, many of them showing the metaphase of the second division, and some having both plates in one plane, with the chromosomes well spaced and stained a deep bluish red, while the cytoplasm is unstained. It takes certainly a modicum of patience to acquire skill with this, as with most microscopical methods.

JOHN BELLING.

STATION FOR EXPERIMENTAL EVOLUTION,
COLD SPRING HARBOR, N. Y.

INDEX

NAMES OF CONTRIBUTORS ARE PRINTED IN SMALL CAPITALS

ALTENBURG, A., Interference in Primula Sinensis, 78.
ANDREWS, E. A., American Folliculinas, 347.
Antherophagus ochraceus Mels in the Nests of Bumble Bees, T. H. FRISON, 188.

Barley, F_1 Species Cross in, W. S. MALLOCH, 281.
Bassler, R. S., and F. Canu, Early Tertiary Bryozoa, A. ROBERTSON, 69.
BATESON, W., Genetic Segregation, 5.
BELLING, J., Counting Chromosomes, 573.
BLAKESLEE, A. F., Types of Mutation, 254.
BRIDGES, C. B., Gametic and Observed Ratios in Drosophila, 51.
BRUES, C. T., Taxonomic Affinities and Food Habits in Hymenoptera, 134.
BURLINGAME, L., Variation and Heredity in Lupinus, 427.

Chiton, Homing Behavior in, W. J. CROZIER, 276.
Chromosomes, Counting, J. BELLING, 573.
CLAUSEN, R. E., and T. H. GOODSPEED, Growth and Nuclear Division in a Parasitic Infusorian, R. W. Hegner and H. F. Wu, 328.
COLLINS, G. N., First Generation Hybrids, 116.
CROZIER, W. J., Homing Behavior in Chiton, 276.

DETLEFSEN, J. A., Mutation in the House Mouse, 469.
Drosophila, Gametic and Observed Ratios, C. B. BRIDGES, 51; Orange Eye Color in, F. PAYNE and M. DENNY, 377; Bar-eye, Sexual Dimorphism in, C. ZELENY, 404; Cross-over Values in, J. D. IVES, 571.
DURHAM, G. B., Inheritance of Belting Spotting in Cattle, 476.

Ears, Short, Mutation in the Mouse, C. J. LYNCH, 421.
Egg-secretion, The Duality of, O. GLASER, 368.

FASTEN, N., Parasitic Copepods, 449.
Fiddler Crabs, Intersexes in, M. J. RATHBUN 80; Juvenile, Variation in, T. H. MORGAN, 82.
Folliculinas, American, E. A. ANDREWS, 347.
FRISON, T. H., Antherophagus ochraceus Mels in the Nests of Bumble Bees, 188.
FROST, H. B., Somatic Segregation, 461; Genetic Terminology, 567.

Generic, Names in Zoology, W. L. MCATEE, 89.
Genetic, Segregation, BATESON, 5; Form and Nomenclature, C. C. LITTLE, 175; Factors in Blending Inheritance, G. H. SHULL, 556, 566; W. E. CASTLE, 564; Terminology, H. B. FROST, 567; W. A. LIPPINCOTT, 570.
GLASER, O., The Duality of Egg-secretion, 368.
Growth, Rate of, H. S. REED, 539.
GUYER, M. F., Immune Sera, 97.

HANCE, R., Chromosomenzahl von Zea Mays L., Y. Kuwada, 268.
HANNA, G. D., Genital Organs of Hermaphroditic Fur Seals, 473.
HARTLEY, C. P., and H. S. GARRISON, Reproducing Power of Maize, 184.
HEGNER, R. W., and H. F. WU, Growth and Nuclear Division in a Parasitic Infusorian, 335.
HIDEN, R. B., Yolk Mass in Oviduct of a Hen, 373.
HUBBS, C. L., Unilateral Reactions of the Melanophores, 286.
Hybrids, First Generation, G. N. COLLINS, 116; Vigor of, D. F. JONES, 457.
Hymenoptera, Taxonomic Affinities and Food Habits of, C. T. BRUES, 134.

Immune Sera, M. F. GUYER, 97.
Inheritance, of Blue in Poultry, W. A. LIPPINCOTT, 289; of Belting Spotting in Cattle, G. B. DURHAM, 476; of Cancer, L. LOEB, 510.
Interference in Primula Sinensis, A. ALTENBURG, 78.
Internal Secretions in Amphibians, E. UHLENHUTH, 193.
IVES, J. D., Cross-over Values in Drosophila, 571.

JONES, D. F., Vigor of First Generation Hybrids, 457.

KJERSHOW-AGERSBORG, W. VON, Melibe Leonina (Gould), 222.
KUWADA, Y., Chromosomenzahl von Zea Mays L., R. Hance, 268.

LEWIS, P. A., and S. WRIGHT, Resistance of Guinea-pigs to Tuberculosis, 20.
Life, Duration of, RAYMOND PEARL, 481.
Linkage in Mice, C. WACHTER, 412.
LIPPINCOTT, W. A., Inheritance of Blue in Poultry, 289; Genetic Terminology, 570.
LITTLE, C. C., Genetic Form and Nomenclature, 175.
LOEB, L., Inheritance of Cancer in Mice, 510.
LYNCH, C. J., Short Ears an Autosomal Mutation, 421.

MCATEE, W. L., Generic Names in Zoology, 89.
Maize, Reproducing Power of, C. P. HARTLEY and H. S. GARRISON, 184.
MALLOCH, W. S., F_1 Species Cross in Barley, 281.
Melibe Leonina (Gould). W. VON KJERSHOW-AGERSBORG, 222.
Microdissection, Apparatus for, G. H. BISHOP and C. E. THARALDSEN, 381.
Microphotography, Standardized, A. PETRUNKEVITCH, 178, 477.
MORGAN, T. H., Variation in Juvenile Fiddler Crabs, 82.
Mutation, Types of, A. F. BLAKESLEE, 254; Autosomal, C. J. LYNCH, 421; in the House Mouse, J. A. DETLEFSEN, 469.

Neuromotor Apparatus of Paramecium, C. W. REES, 464.
Nomenclature, W. L. MCATEE, 89; C. C. LITTLE, 175.

OSGOOD, W. H., Turkey for Experiment, 84.

Parasitic Copepods, N. FASTEN, 449.
PATTERSON, J. T., Sex-ratios in Platygaster, 180.
PAYNE, F., and M. DENNY, Orange Eye Color in Drosophila, 377.
PEARL, RAYMOND, and S. L. PARKER, Duration of Life, 481.
PETRUNKEVITCH, A., Standardized Microphotography, 178, 477.
Plants, Body and Organ Size in, E. W. SINNOTT, 385.
Polyembryony in the Armadillo, C. R. STOCKARD, 62.

RATHBUN, M. J., Intersexes in Fiddler Crabs, 80.
Reactions, Unilateral, of the Melanophores of the Head in Fishes, 286.
REED, H. S., Rate of Growth, 539.
REES, C. W., Neuromotor Apparatus of Paramecium, 464.
RENSHAW, R., Yeast and the Utilization of Food, 73.
ROBERTSON, A., Early Tertiary Bryozoa, F. Canu and R. S. Bassler, 69.

Seals, Hermaphroditic, Genital Organs of, G. D. HANNA, 473.
Segregation, Genetic, W. BATESON, 5; Somatic, H. B. FROST, 461.
Sex, Transformation of, and Gonads in the Frog, E. WITSCHLI, 529; -ratios in Platygaster, J. T. PATTERSON, 180.
SHULL, G. H., Genetic Factors in Blending Inheritance, 556, 566.
SINNOTT, E. W., Body and Organ Size in Plants, 385.
STOCKARD, C. R., Polyembryony in the Armadillo, 62.
Sucking Fish, L. WIENER, 165.

Tuberculosis, Resistance of Guinea-pigs to, P. A. LEWIS and S. WRIGHT, 20.
Turkey for Experiment, W. H. OSGOOD, 84.

UHLENHUTH, E., Internal Secretions in Amphibians, 193.

Variation and Heredity in Lupinus, L. BURLINGAME, 427.

WACHTER, C., Linkage in Mice, 412.
WIENER, L., Sucking Fish, 165.
WITSCHLI, E., Gonads and Transformation of Sex in the Frog, 529.
WRIGHT, S., and P. A. LEWIS, Resistance of Guinea-pigs to Tuberculosis, 20.

Yeast and the Utilization of Food, R. R. RENSHAW, 73.
Yolk Mass in the Oviduct of a Hen, R. B. HIDEN, 373.

ZELENY, C., Sexual Dimorphism in Bar-eye Drosophila, 404.